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The social bee
John Free reviews Charles Michener's
book 'The Social Behaviour of the
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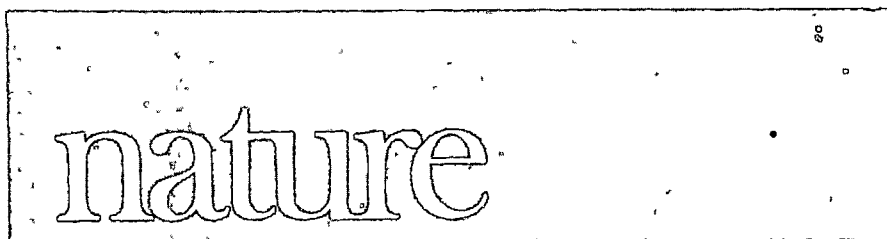
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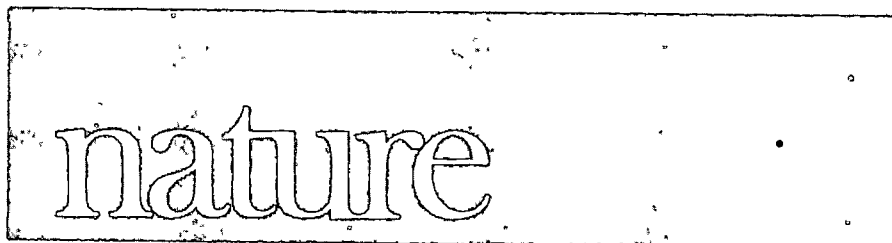
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Cover Picture

'Kits Coty House', a megalithic monument near Aylesford, Kent. On page 431, a group of astronomers reports a survey of three other megalithic sites in Argyllshire Scotland (Mary Evans)

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Series editor. H. Tajfel

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The Illusion of Attitude Change

Toward a Response Contagion Theory of Persuasion

J. M. Nuttin, Jr.

February 1975, approx. 220 pp, £6 40/\$17 00

0 12 522940 2

This monograph focuses on a progressive and systematic theoretical analysis of some dramatic shifts in the attitudes of university students towards a very popular university reform issue. The author believes that the results of this analysis show the classical social attitude concept to be misleading for both the fundamental and applied study of social behaviour.

Contents: The Cohen-Rosenberg controversy and our 1964 research. An experimental challenge of the interpretation of a cognitive dissonance effect. Evidence for an a-cognitive 'dissonant' view. The illusion of past-advocacy attitude change. An evaluative response contagion view of persuasion. Conclusion. Appendix. References. Author index. Subject index.

Published jointly with Leuven University Press

The Biology of Agricultural Systems

C. R. W. Spedding

February 1975, x+262 pp, £7 40/\$19 50

0 12 656550 3

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The work discusses the methods available for identifying the relevant biological sub-systems of agriculture as a basis for research, and examines the role of research in developing agricultural biology.

Phytochemical Society Symposia Series Number 11

Proceedings of the Phytochemical Society Symposium, Ghent, Belgium, 1973

The Chemistry and Biochemistry of Plant Proteins

edited by J. B. Harborne and C. F. Van Sumere

February 1975, xiv+326 pp, £11 60/\$30 75

0 12 324668 7

Over recent years there has been little literature devoted specifically to the protein of higher plants, although much has been written about proteins in general, and it is this gap which the present volume is intended to fill.

Contents: Amino acid sequence analysis of plants. Immunochemical investigations of plant proteins. Properties of the physiological changes in storage proteins. The proteins of barley. Mechanism of protein synthesis in higher plants. The biogenesis of plant mitochondria. The biogenesis of chloroplasts. Plant proteins and phenolics. Protein sweeteners. Proteins and taxonomy. Author index. Subject index.

Plants Consumed by Man

B. Brouk

February 1975, approx. 450 pp, £14 80/\$39 25

0 12 136450 X

The aim of this book is to provide a comprehensive survey of plants for human consumption, including all primary food plants, those which yield non-nutritive ingredients such as flavours, colours, thickening agents and the bacteria and fungi which produce edible materials or which are themselves consumed. Also included, is a special section on plants used for smoking and chewing.

Contents: Introduction. Cereals and Pseudo-cereals. Vegetables. Fruits. Nuts. Plant extracts. Flavouring plants. Beverage plants. Fumitories and masticatories. Fermentative micro-organisms. Morphological survey of plants consumed by man. Glossary (An explanation of morphological terms used in the present book). Selected literature. Index.

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edited by R. D. Martin

February 1975, approx. 450 pp., £12 80/\$33 75

0 12 474850 3

This book is the result of the first international conference on the planned breeding of endangered species in captivity, held in Jersey in May 1972. The papers in this collection have been extensively re-edited and re-written in the interim period. The meeting was sponsored by the Fauna Preservation Society and the Jersey Wildlife Preservation Trust.

This extensively illustrated work is the first to deal specifically with the captive breeding of endangered species. With contributions from Sir Peter Scott, Gerald Durrell, Philip Wayre and many distinguished colleagues, it will be of great value to anyone interested in conservation, and it will provide basic reading for zoo curators, academic staff and professional conservationists.

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Opium poppy harvest in Turkey
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Japanese bullet train
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port systems on page 580
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Cover Picture

A drawing of common purslane from
Rheede's *Horius Malabaricus* of 1690
*Was purslane in America before 1492?
See p 726

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nature

January 3, 1975

Manpower needs are not so easily estimated

IN A DOCUMENT put about by the Manpower Services Commission of the Department of Employment, the claim is made, amongst others, that in the off-shore oil industry 'immediate and continuing shortages of geologists, petroleum engineers, drillers and divers have been established'. The circumstances surrounding the report—*The discovery of off-shore oil and gas: manpower implications*—are such there must be the gravest doubts that it merits the extensive publicity it has so far received.

The commission, about which you will learn nothing at all on any page (or the cover) of the report—not even its address, telephone number or where it fits into ministerial structure, sponsored Mr David Begg of Nuffield College, Oxford to write a report which could be an overview of manpower implications of the discovery and exploitation of off-shore gas and oil. Mr Begg took only a couple of months over the draft and has now left to do postgraduate research in the United States. Subsequently Mr John Fyfe, of the commission's secretariat, 'up-dated, revised and expanded' the draft, but in publishing it (at least I suppose it's published although it has 'confidential' on the front page) the commission made the odd statement that the views were those of the authors and not necessarily those of the commission. It is mystifying to know why a report is being disseminated for 'interest and assistance' if the commission cannot stand behind it.

Mr Begg seems to have consulted and drawn extensively from about ten more specialised studies in his overview; it is unclear, however, how many people in the industry he spoke to. The result, as one critic put it, is an A+ for the college essay prize but no marks for producing a document of any help to policymakers in deciding what, if anything, should be done by government, industry and educational authorities.

The authors draw heavily on figures from the Petroleum Industry Training Board (PITB), also a Department of Employment agency. PITB forecasts for oil-related employment are based on a projected number of mobile drilling rigs which, the authors admit, was demonstrably in error as early as June 1974. These errors may partly spring from the nature of a multinational, ever-changing industry in which are all sorts of service companies which do not report operations and employment figures to the board. To go on to accept PITB projections on employment merely with the comment that they should be

treated as conservative estimates is a poor substitute for the detailed investigative work that is needed.

As it is, the report, echoing the PITB, sees the number of jobs for geologists/geophysicists rising from 440 in 1973 to 520 (misprinted as 570) in 1980; for petroleum from 190 to 330 in the same period, and for divers from 300 in 1974 to 650 in the late 1970s.

Can skilled men be provided in sufficient numbers? The report, supposedly aimed at this question, never really answers it at all. Amongst geologists, for instance, what evidence is there that there is a shortfall on the 200 that the PITB say can be employed at present? Or that there will be a shortfall on the 220 that could be used in 1980? None at all, and the authors never address the problem, instead tut-tutting that university geology courses are unsuited to the demands of petroleum geology. If the authors instead of rushing into print had chosen to talk to some of the major oil companies, they would have found that the companies run extensive training courses and prefer the unpre-doctrinated graduate. They would also have found that the companies are acquiring geology graduates without difficulty in what one executive described as a buyer's market. And yet the report speaks of having 'established' an immediate and continuing shortage of geologists.

A similarly critical analysis could probably be applied in the other professions. There seems to be an all-too-ready desire to call any differential between present and future needs a shortfall without a serious examination of ways in which the oil industry is likely to respond. And since the industry is multinational, professionals that can't be found in Britain will probably be imported.

In the case of deep-sea diving, where skills will undoubtedly be in demand for many years, the authors choose to use the emotive turn of phrase rather than careful study. Perhaps 'inadequate training and a high fatality rate' is a correct summing-up of the scene, but what confidence can one have that this undocumented charge is anything more than much else in the report—hunch and hearsay?

It may well be true that there will be shortages of skills in the North Sea, but it would be dangerous to base any plan of action on the evidence of this report. The ability of the British government to monitor what is happening under its own nose must be brought into the most serious doubt by this document. □

THE newly formed United Kingdom section of the International Solar Energy Society has set itself a formidable task in the first year of its existence. It is preparing on its own initiative an assessment of the potential usefulness of solar energy in Britain, along the lines of reports recently prepared in Australia and the United States.

A working party of some forty experts in the various branches of solar energy use has been set up with the help of a grant of £5,000 from the Wolfson Foundation under its programme to support research into the better use of Britain's natural resources, and the report on solar energy should be published in September 1975.

Although the outlook for solar energy in the gloomy days of a British winter seems on the face of it distinctly bleak, a measure of the interest in its possibilities, even in northern latitudes, is given by the rapid growth of the UK section since its formation early in 1974. It now boasts a membership of more than 450, second only to the North American section.

A definite check to research into the applications of solar energy in Britain was given earlier this year by the Central Policy Review Staff, the Think Tank, which recommended in its report on energy conservation that it would not be useful to increase government support for such research. One of the aims of the Solar Energy Society's report will undoubtedly be to try to persuade the government otherwise. By definition the members of the society believe that solar energy has a future in Britain in the short term, in the long term as a source of inexhaustible energy when fossil fuels are exhausted, and as one alternative to the large scale use of nuclear power. The problem as they see it is to pinpoint the areas of technology and basic research which can be most usefully developed to serve the special needs of a highly industrialised, densely populated country like Britain, with a climate which is far from ideal.

But there are ways in which solar energy could make an increased contribution to Britain's energy needs fairly soon, in providing supplementary water and space heating. The technology is available and there is already a small commercial solar heater industry in Britain. With the costs of gas and electricity all set to rise to 'realistic' levels, which will mean an eventual rise of some 30% in electricity prices, supplementary solar power, plumbed into existing hot water systems and suitably insulated, for water heating could become very attractive if it were relatively cheap and easy to install. One of the problems the report will have to consider in this context, how-

ever, is the feasibility of installing this sort of heater in existing buildings.

With regard to the longer term ideal of designing new houses and institutional buildings to take more account of solar radiation and perhaps incorporate solar heating devices, economics and social acceptability will probably

Solar energy in Britain



Solar collectors for heating

The UK Section of the Solar Energy Society has got off the ground with a bang. Eleanor Lawrence has been talking to some of its members about the report on the potential of solar energy for Britain now in preparation.

be the main barriers, although there are still technical problems to be evaluated and overcome.

Many of the other applications of solar energy to be considered in the report are far more speculative. Solar energy research is nothing if not multidisciplinary, and architects and engineers rub shoulders with plant breeders and photobiologists. Green plants have solved not only the problem of capturing sunlight but also the vexed question of how to store the energy derived from it, a problem which is currently exercising the brains of everybody involved in the utilisation of intermittent and irregular sources of power.

The section of the working party dealing with agricultural and biological systems will consider plants both as a source of materials, including food, and as a source of fuel. Research is already in progress in Britain to breed and select plants which make a better use of the available solar energy through a longer growing season and an extended range of growing conditions. As to fuel, the fossil kinds represent a non-renewable source of solar energy stored by plants. So, the argument goes, why not use

plants as a renewable source of fuel by converting plants and plant wastes to liquid fuels, gas and char by pyrolysis and fermentation. Problems the report will have to consider here are the availability of suitable plants, land and the technology needed for making use of the products. Plants grown for fuel may not necessarily have to compete for valuable land with plants grown for food, and it is here that research on the better use of available crops and the introduction of previously unconsidered types as food and/or fuel will be of prime importance. One of the main difficulties with any increase in the growing and harvesting of plants for whatever purpose will be the increased amounts of fertilisers needed. A major limiting factor is expected to be the availability of phosphate, a non-renewable resource produced by only a few countries. Recycling of phosphate and other non-renewable fertilisers is therefore an essential prerequisite before any such schemes can be put into operation. In the report prepared by the Australian Academy of Sciences, the long term nature of such work and the necessity for an early start on research was emphasised, a plea echoed by Professor David Hall, of the Department of Plant Science at King's College London, Chairman of the Agriculture and Biological Systems Section of the working party. If funds for such research are not made available very soon so that the problems and potentials can be properly identified, any useful contribution will take even longer to materialise than the twenty years envisaged by Professor Hall.

Similar considerations apply to work on the photochemical and photobiological conversion of solar energy. The study of the photosynthetic apparatus with the eventual aim of developing synthetic or semi-synthetic systems for using solar energy to split water to produce hydrogen in large amounts, say, has enormous long term implications but is threatened in the present times of financial stringency.

There is a greater industrial interest in photovoltaic solar cells because experience has been built up as a consequence of the space programme, but present terrestrial applications are limited to a few specialised uses as at present costs are very high. If components suitable for mass production could be developed costs would soon fall to economic levels opening up new possibilities for the generation of electric power.

Even if it eventually transpires that the potential for solar energy is indeed fairly limited in Britain—the Society's committee remains open-minded on this issue—Britain can hardly afford to miss out on the potentially valuable export market in solar technology. □

international news

EIGHT THOUSAND million francs in the French budget for scientific research means a growth of 13% over 1974—barely enough to keep pace with inflation. It has been described as a marking-time budget, and hardly more could have been expected in the present economic climate. Moreover the new government, coming after a long period when no decisions were taken, has not yet put forward a definitive policy on all aspects of science. Bernard Gregory, director of the Centre National de la Recherche Scientifique (CNRS), recently summed it up in an interview in *Le Monde*, "it won't do much harm to future prospects as the money has been distributed with a view to keeping open all options but it is not a situation which can go on for many years".

The *enveloppe recherche*, as the budget is called, is only concerned with interministerial projects (which fall within the province of the Délégation Générale à la Recherche Scientifique et Technique (DGRST)). Military, telecommunication and aeronautics expenditures are the concern of their own ministries (in 1973 they came to 6,230 million francs). In 1967 2.2% of GNP was devoted to all research, now it is 1.7%. The increase in public expenditure between 1969 and 1973 was only 33%, compared with 69% in Italy and

Changes due in French science

from the staff of La Recherche

95% in Germany.

There will be very few new jobs, this is of particular concern to government laboratories for which the budget gives authority to take on permanently people of specific skills. In 1971, 432 research jobs were created, in 1973, 200, for 1975 only 156 are projected. The Minister of Industry and Research M d'Ornano, criticised for this running down of manpower, replied that one should add to this figure 194 technicians and 264 *vacataire* posts (research workers on short term contracts).

A controlled budget means a restriction on non-current expenditure for new projects. The public sector has not suffered too much in this way—construction of new laboratories or purchase of new equipment can be postponed—but private laboratories living on state funds will feel the pinch, many scientists could find themselves out of work. This was already happening in mid-1974 in the social sciences sector.

There are priority areas, mainly in

the fields of production and distribution of energy. After several stagnant years, the atomic energy commission starts to grow again by 26.7% in current francs. Most of this will go to reactor safety, treating of fuel elements and the storage of nuclear waste. The rest of the energy expenditure (up to F178.4 millions from F116.1 millions in 1974) will go mainly on thermonuclear fusion research and to a lesser degree on energy conservation, solar energy (F12 million) and geothermal energy (F5 million). Nobody contests the high priority for energy, even so, some deputies have qualms about the overwhelming and unthinking emphasis on electricity production.

Other priorities are biology and certain parts of the social sciences. Research with socio-economic ends (environment, urbanism, transport, pollution) still grows but less than it did in previous years. After much hesitation the launcher *Ariane* is kept thanks to deft budgetary transfers and since *Ariane* is interlocked with other projects, space is relatively well supported financially.

Fundamental research, however, as practised at CNRS and in the universities stagnates, there will only be a 7% growth in current francs, and this is causing much worry.

IN France, as in most western countries, the role of scientists in determining the major issues of science policy has been declining since the late 1960s. The large research institutions are, it is true, in general directed by scientists, but these people are so deeply involved in management that they act more as scientific administrators than as practising scientists. The 1958 reforms in these institutions led to the creation of two key bodies, the Délégation Générale à la Recherche Scientifique et Technique (DGRST), having an interministerial coordinating role in science policy under the Prime Minister and the Comité Consultatif de la Recherche Scientifique et Technique charged with advising the government on matters of research policy. This latter consists of a dozen members, *les douze sages* nominated by the government for periods of four years. *Les douze sages* was a lively and important body to start with. In particular, it prepared the research budget in liaison with the DGRST, and although its advice was not always followed it had an active

Wise but powerless

from the staff of La Recherche

role in the first years of the Fifth Republic, a time when many research bodies were constituted or reformed.

French science policy changed after 1965 under M Pompidou. The priority that research had possessed early in the Fifth Republic was no longer there, and ministers for research after 1965 had less political clout than their predecessors. The influence of *les douze sages* waned and that of administrators grew accordingly.

At present the committee has practically no influence and now that the government has announced a reform of science policy machinery, and particularly of the coordinating body of the DGRST, the role of the committee has come into question. The Minister for Industry and Research, M d'Ornano, said in a recent debate on the research budget in the National Assembly "maybe it is time to start those who are involved in research policy thinking about a problem which

has so far passed them by but to which they should not be indifferent—that of society's control over technological development. One of the first things we could do is bring non-scientists, elected politicians and consumer representatives into closer touch with the implementation of new technologies and the definition of new programmes". The president of *les douze sages*, M Brams, has received the mandate to think about possible reform of the committee and to make proposals relevant to d'Ornano's plan. The problem is that of non-scientists being involved in science policy decisions and this issue had already been aired in Mitterand's presidential campaign, when the idea had been put forward of a high ranking consultative committee on science policy whose members would include non-scientists.

The big question is, if twelve becomes fifteen or twenty will the scientific community and even the whole nation be better represented and will the committee have more power and authority than at present?

NIH Director edged out

by Colin Norman, Washington

FOR the second time in two years, the Director of the National Institutes of Health (NIH) has been fired, apparently because of disagreements with other key administration officials over how biomedical research should be managed and funded in the United States.

Last month, Dr Robert S. Stone, who had been NIH Director since May 1973, was asked to resign by Dr Charles C. Edwards, the top health official in the Department of Health, Education and Welfare (HEW). The event immediately evoked a chorus of protests from several prominent biomedical scientists who charged in a statement put out by the Federation of American Scientists that the NIH is being subjected to "unwarranted and counterproductive political control".

Although no official reasons have yet been given, Stone's downfall is widely believed to have resulted from his opposition to further cutbacks in the NIH's budget and also from his failure to see eye-to-eye with Edwards on the role that the NIH should play in the total national health care programme. In any case, the troubles between Stone and the top brass at HEW (of which the NIH is officially a part) are symptomatic of the widespread disquiet within the biomedical research community over the administration's policies for the NIH.

The disquiet stems chiefly from the

fact that the autonomy which the NIH has traditionally enjoyed has been considerably eroded in the past few years. Until the late 1960s there was little scrutiny of the NIH's programmes either by congress or the rest of the administration, but in the past couple of years, there has been a move to exert considerable management control over the NIH from Edwards's office in HEW. In addition the budget for biomedical research has levelled off and there has been considerable redistribution of funds from several areas of research into the politically sensitive cancer programme.

But the chief bone of contention came early last year when the administration proposed to scrap the NIH's training and fellowship programmes. Dr Robert Q. Marston, Stone's predecessor as Director of the NIH, was in fact fired when he resisted that proposal. After that, the administration let it be known that it was considering some radical surgery on the NIH's peer review system. Although both those moves have since been thwarted, largely because of loud protestations from biomedical scientists, Stone's forced departure is being viewed as yet another move towards tighter political control over the NIH from bureaucrats in HEW.

In an unrelated move, Dr Edwards himself resigned from the government to take up a post in industry a week after Stone's resignation was made known. Two top health posts are therefore vacant and the biomedical research community is anxiously waiting to see who will fill them.

University of Ghana celebrates jubilee

DURING the week (November 30 to December 7) the University of Ghana, situated in the Accra suburb of Legon, held its silver jubilee celebrations. In true academic style, the university had arranged lectures and exhibitions as well as two graduation ceremonies—one for the normal crop of last year's graduates and one for the presentation of honorary degrees to distinguished luminaries, including Dr Conor Cruise O'Brien, former Vice-Chancellor of the university.

The silver jubilee celebrations at Legon, like the recent silver jubilee celebrations at the Nigerian University of Ibadan, are certainly justified, for both universities have played a vital role in producing the trained manpower needed to run a newly emergent state. Sometimes the training process has not run particularly smoothly. Legon's celebrations were actually to have been held

last March but student demonstrations led to a closure of all three Ghanaian universities and to the cancellation of the original silver jubilee celebrations for Legon. Dr Alex Kwabong, the University of Ghana's skilful Vice-Chancellor has managed to keep the situation quiet this academic year, however, and the university's stock has risen appreciably since March. Kwabong is a masterful negotiator and diplomat whose leadership and individualistic style, based on the groundwork laid by his predecessors, have helped to secure international prestige and financial aid for the campus.

Although all Kwabong's subordinates may not have his flair for administration, there has been significant growth in the university. The University of Ghana Medical School, which simultaneously celebrated its tenth anniversary, recently started dental and postgraduate courses. In addition, various other programmes such as computer science, journalism and population dynamics have all been set up within

the past two years.

The University has, in the past, been fortunate in attracting distinguished academics and promising young graduates to fill its teaching and research posts. This is, in part, a result of the generosity of the British, Canadian and Dutch governments who provide finance and high level manpower through technical assistance schemes. It is difficult to find Ghanaians of the appropriate calibre to man the nation's universities. The pecuniary rewards overseas, especially in the United States, are so enticing that Ghana, in common with all developing countries, suffers from a severe brain drain.

Overall the university academic staff is 75% Ghanaian but this proportion varies greatly from faculty to faculty.

In the scientific disciplines, where 41% of the professors and lecturers are non-Ghanaians, the loss of promising Ghanaians is most acute. Their absence abroad is most unfortunate, since the high quality of Legon's scientific research was documented in a recent study of the faculty of science funded by the Ford Foundation. The Ford Foundation's report also suggested a vigorous infusion of money and equipment to maintain the existing high standards. Unfortunately, in the present bleak economic climate, no benefactor has as yet been found to fulfil the committee's recommendations.

In a shrewd display of foresight, the Ghanaian government launched "Operation Feed Yourself" in 1972 in an effort to achieve self-sufficiency in food production. The faculty of agriculture met this challenge by expanding its research programme to improve the varieties of tropical rice and sugar cane being grown in Ghana. As a peripheral benefit the expanded production on the research farms has helped both the university, which can now economise by feeding its students some home-grown agricultural produce, and the Legon housewife, who can now buy hitherto unobtainable goods such as pasteurised milk, frankfurters and liver sausage.

Contemplating Legon's success in the past twenty-five years leads to great hopes for the next twenty-five. It is no mean achievement to teach well and to carry out good quality research in a developing country subject to an acute shortage of foreign exchange delays in imports, low indigenous salaries, a rate of inflation of 20% and a lack of technical staff, and where the whole economic climate revolves around the vagaries of a single agricultural commodity—cocoa. The ability to surmount these obstacles speaks well for the dons at Legon who, in the words of the university's motto, have achieved "progress with integrity". □

SIR,—A letter from Gerschenfeld and others concerning the problems of Chilean scientists under the military rule (*Nature*, March 29, 1974) has been answered by Eyzaguirre (*Nature*, July 5, 1974). I feel it my duty to present the view of a Chilean scientist, a witness of the entire process who works in Chile and intends to stay.

Since I do not think *Nature* to be the proper place for a political argument I shall only say that I disagree entirely with Eyzaguirre's interpretation of what happened in Chile. But neither can I accept his statements of facts. I do not deny the data—which refer to the Catholic University—the problem being rather that he would have others believe that the case of the Catholic University is representative of the Chilean universities. Without saying so explicitly, Eyzaguirre conveys that impression by mixing data of restricted significance with a very general and in my opinion superficial assessment of the Chilean situation before, during and after the military takeover.

According to Eyzaguirre, in the Catholic University "only 3.25% of faculty members were removed after the coup." The information I have collected for the University of Chile shows that approximately 30% of the faculty members and 11% of the non-academic staff were dismissed after the coup. The significance of this information stands out when one considers that the University of Chile enrolls 50% of the university students compared with 8% enrolled by the Catholic University.

I have been unable to obtain reliable data concerning the State Technical University, where the military intervention was most drastic. As to the University of Concepción (12.3% of the student population) the information I have points to at least 30% of faculty members dismissed. This figure is also a fair estimate for the Austral University (2% of the student population). It has been asserted that the worst hit have been the social sciences, but the private Technical University Santa María (3.3% of the student population) lost about 25% of its faculty members. I do not have dependable information on the Catholic University of Valparaíso (5.2% of the students) and the University of el Norte (4.2% of the students).

To sum up, the universities that enrol 75.6% of the students have lost about 29% of their faculty members since the military intervention, that is approximately 5,000 people, but dismissals still continue. As to the universities on which I have no consistent data (24.4% of the student population) the percentage of faculty members removed is presumably higher, consider-

ing that the State Technical University was entirely reorganised and is the second largest university in Chile.

The percentages of faculty members dismissed up to now are estimates based on various sources and might require some correction if reliable information is eventually disclosed to the public. Nevertheless, I trust that my estimates—especially those for the University of Chile—would stand a fair test and anyhow they clearly prove that the Catholic University is an exceptional case.

As to how many of the 130,000 university students have lost their

Letter from Chile

Dr Luis Izquierdo of the Faculty of Sciences at the University of Chile describes the situation now obtaining in the Chilean universities

places, dependable information is unavailable at this stage. On the one hand it is still uncertain how many might be allowed to register again, on the other hand, more than one year after the military intervention, students are still being expelled.

Eyzaguirre claims that faculty members removed from the Catholic University had been "engaged exclusively in different forms of political activity." Of course, political activity was not forbidden before the military intervention and nobody should be retrospectively condemned unless proven that he or she was exclusively engaged in these activities, since it is not the role of universities to support full time professional politicians either from the right or left. But, have there been thorough investigations?

It might be again the exceptional case of the Catholic University and hence the low percentage of faculty members removed. It is certainly not the case of the University of Chile in which faculty members were summarily investigated on the basis of secret denunciations. Charges were unspecified and all the defendant could do was to ask for certificates of good conduct from his less scrutinised friends. The procedure has been so irregular that in practice all decisions rested in the "fiscals" appointed by the military rector. I have witnessed much injustice, persecution and vindictiveness. I also must admit that some "fiscals" accepted their role in this legal farce so as to bring some help to their colleagues in disgrace.

In the State Technical University, University of Concepción and Austral University there was not even the

pretence of a legal procedure. The resolutions were based on black lists and faculty members or students were given notice through a standard form which specified no charges.

A decree from the government forbade universities and public institutions to employ faculty members dismissed, so those who had been engaged in research and teaching for years—notably in the basic sciences—had no alternative but to emigrate if they cared to persevere in their work. How can Eyzaguirre decide for practising *gauchisme de salon* those who left Chile and those lending them a hand from abroad? Of course there are people who have taken advantage of the international benevolence and claimed persecution when really looking for a better job, but they are exceptions and the overwhelming majority could not remain here because they had lost their jobs or felt threatened or could not endure the repressive environment.

The percentage of faculty members dismissed does not show in all its magnitude how serious the loss is for science in Chile. Many more scientists have left because of difficulties encountered in their work, such as disruption of research groups when senior investigators were dismissed, lack of research funds or of up to date libraries. The Chilean Society of Biology includes in its 414 members almost all the biologists at least partially engaged in research, 28% have left not to return under the present conditions. The situation of the Faculty of Sciences of the University of Chile is even worse. After struggling for 10 years we had succeeded in establishing it on a firm basis. Now, of the 123 faculty members in the department of Mathematics, Physics, Chemistry and Biology, 60 have left the country. Some would probably have been dismissed had they stayed, others lost hope of pursuing their research work at a satisfactory pace. Those of us who have invested so much of our time in the organisation of this Faculty, bitterly resent a set-back that takes us again to that state of dependence on foreign countries which we had sought to overcome.

The military intervention in Chilean universities has been up to now mainly repressive and has not achieved a new order. Recognised age-old vices of our university system persist in spite of the almost absolute power of present military rectors.

I have written this letter for the sake of truth to the best of my knowledge, convinced as I am that innuendoes, slander and lies from many quarters have corrupted Chilean political life in the last decade causing much individual misery and social distress.

news and views

Towards an anti-viral vaccine for a human cancer

from M A Epstein

WHEN the US National Cancer Institute set up the Special Virus-Leukaemia Program in 1964 (later expanded as the Special Virus Cancer Program) with the explicit objectives of (1) determining whether at least one human cancer is caused by an oncogenic virus, and if so of (2) developing an effective vaccine for the control of such a tumour (Rauscher, Carrese and Baker, *Cancer Res*, **26**, 1176, 1966), the project was viewed with considerable scepticism by many leading workers in the cancer and virus fields. Looking back at the impressive progress made in tumour virology over the last ten years, the far-sightedness of the whole project is now evident, particularly in relation to the herpesviruses following striking recent advances with vaccines against some oncogenic members of the group.

Herpesviruses are known to cause the ubiquitous Marek's type of malignant lymphoma in chickens (Churchill and Biggs, *Nature*, **215**, 528, 1967; Solomon *et al.*, *Proc Soc exp Biol Med* **127**, 173, 1968), an experimental malignant lymphoma in South American sub-human primates (Meléndez *et al.*, *Lab Animal Care*, **19**, 378, 1969), and the Lucké kidney carcinoma of the leopard frog (Mizell *et al.*, *Science*, **165**, 1134, 1969; Naegele *et al.*, *Proc natn Acad Sci USA*, **71**, 830, 1974), and the closeness of the association of Epstein-Barr virus with African Burkitt's lymphoma and nasopharyngeal carcinoma in man is increasingly suggestive of some aetiological relationship (Epstein and Achong, *A Rev Microbiol*, **27**, 413, 1973; Klein in *The Herpesviruses*, edit by A Kaplan, Academic Press, London and New York, 1973; Henle and Henle, *Cancer Res*, **33**, 1419, 1973). There is also a more tenuous association between herpes simplex virus type II and carcinoma of the human cervix (reviewed by Rapp and Buss, *Am J Pathol*, **77**, 85, 1974), but this has been weakened by the failure of biochemical probes to detect the viral genome in tumour biopsy cells (zur Hausen *et al.*, *Int J Cancer*, **13**, 657, 1974).

The development in 1969 of live, attenuated herpesvirus vaccines capable of giving chickens almost complete

protection against Marek's lymphomas (Churchill *et al.*, *Nature*, **221**, 744, 1969; Okazaki *et al.*, *Avian Dis*, **14**, 413, 1970) was of great significance both in economic terms and because it provided the first example of a naturally occurring malignant tumour to be controlled in this way. Although the importance of this step forward was widely recognised, its applicability to the ultimate control of those human cancers suspected of having a herpesvirus cause was clearly slight because of the impossibility of administering to man a suspected tumour-inducing virus, however attenuated. Indeed, it is unlikely that even an inactivated virus of this kind would ever be usable as a human vaccine because of the difficulty of proving total inactivation and the possibility that traces of the viral DNA in such a preparation might be capable of bringing about malignant transformation. But further progress with oncogenic animal herpesvirus vaccines is beginning to indicate the ways in which these difficulties could be overcome. Thus, it is now known that chickens can be significantly protected against Marek's lymphomas by vaccines free of virus nucleic acid. Lesnick and Ross (*Br J Cancer*, in the press) have reported some success with a vaccine consisting only of soluble viral antigens extracted from Marek's virus-infected tissue culture cells by treatment with nonionic detergent and other workers have used highly purified plasma membranes from similar virus-infected cells as a vaccine, and reduced the mortality from Marek's lymphomas by 94% when the vaccinated chickens were subsequently challenged with virulent virus (Kaaden and Dietzschold, *J gen Virol*, **25**, 1, 1974).

Now Laufs and Steinke (this issue of *Nature*, page 71) have moved the problem closer to man by showing that sub-human primates can also be successfully protected against a herpesvirus-induced malignant lymphoma. These workers have used a combination of heat and formaldehyde to inactivate herpesvirus saimiri, which is otherwise carcinogenic in South American primates, and have shown by complement-fixation tests that the inactivated vaccine maintains consider-

able specific viral antigenicity after these treatments. Forty-two cotton-top marmosets have been immunised with the vaccine in the present experiments, have remained well after this, and have developed high titres of neutralising and complement-fixing antibodies to the virus. So far 22 immunised animals have been challenged with filtered, virulent herpesvirus saimiri in doses many times greater than those needed to induce fatal lymphomas in control animals and at the time of writing all were alive and well between 121 and 293 days after the challenge, whereas inoculated control animals died of malignant lymphoma within 52 days.

It seems reasonable to anticipate that herpesvirus saimiri vaccines free of viral nucleic acid will be developed before long on similar lines to those already being used to protect against Marek's lymphomas in chickens. The possibility this provides of a model system involving animals phylogenetically related to man has important implications for the planning, production, and testing of vaccines against suspected human tumour viruses. In this connection Epstein-Barr virus is not only a leading candidate because of its special association with Burkitt's lymphoma, but also has certain unique advantages. Thus, as has been pointed out elsewhere (Epstein and Achong, *A Rev Microbiol*, **27**, 413, 1973), since Epstein-Barr virus causes infectious mononucleosis the efficacy of any vaccine developed could be tested by its ability to protect those at risk from this disease, and the existence of areas of high endemicity of Burkitt's lymphoma provide populations where the effect of such a vaccine on tumour incidence could be relatively easily tried out. Finally, since Burkitt's lymphoma occurs most frequently around the age of six, vaccination of infants in the months after birth should allow the efficacy of protection against Burkitt's lymphoma to be judged within five to 10 years. It seems that tumour control by an anti-viral vaccine will be the only way of proving whether or not Epstein-Barr virus is a human tumour virus aetiotogically related to Burkitt's lymphoma either with or without additional cofactors.

Circular polarisation in the Crab nebula

from R D Davies

THE Crab nebula—the remnant of the supernova of AD 1054—is a very remarkable object. It is unique in that it has been detected over the entire electromagnetic spectrum from radio through infrared and the visual to X rays and γ rays. Moreover the pulsar with the shortest known period lies at its centre. This pulsar holds the key to the source of energy in the Crab nebula which had been a mystery before its discovery. The pulsar is a neutron star rotating at 30 Hz. The important property of the pulsar in the context of the energy source of the Crab nebula is that it is slowing down—the rate of loss of rotational energy of the pulsar is equal to the energy radiated by the surrounding nebula.

The next stage is to understand the mechanism by which the pulsar supplies energy to the nebula. This energy is evident in the form of relativistic electrons and magnetic fields as these are required to produce the synchrotron emission from the nebula. But a simple estimate of the lifetime of the relativistic electrons which are responsible for the optical continuum emission indicates that they have a lifetime of only a few years. As a consequence such high energy electrons must be replenished continuously. This point was clearly recognised in the earliest work on the topic. Oort and Walraven in 1956 suggested that the moving wisps of gas seen near the centre of the nebula were evidence of continuing activity from a central star.

Recently Rees and Gunn (*Mon Not R astr Soc*, **167**, 1, 1974) have looked afresh at the problem of the supply of relativistic electrons and magnetic fields to the nebula. They took a theory originally proposed by Piddington in 1957 and discussed subsequently by Kardeshev which had a central object responsible for winding up the magnetic field and thereby maintaining its strength. Rees and Gunn showed that the magnetic flux through the object increases in proportion to the number of rotations of the central pulsar; this indicates that as the nebula ages the magnetic field becomes increasingly important. The configuration of the field will be toroidal with an axis parallel to the spin axis of the pulsar. This axis is taken to lie along the major axis of the nebula (NW–SE). Linear polarisation measurements at optical and radio wavelengths show that the magnetic field is uniform and is essentially perpendicular to the major axis as would be expected from a toroidal configuration if the axis of rotation is in the plane of the sky.

Synchrotron theory indicates that more stringent tests can be made of the field configuration by measuring circular polarisation. Legg and Westfold (*Astrophys J*, **154**, 499, 1968) made a detailed analysis of the polarisation produced in the synchrotron mechanism and showed that a small component of circular polarisation is present. The sense of the circular emission depends on the inclination of the line of sight to the normal to the magnetic field, while the degree of polarisation is proportional to the square root of the magnetic field divided by the frequency. Such circular polarisation has already been detected in the radio emission from the magnetosphere of Jupiter and from several flat spectrum (that is, compact) extragalactic radio sources. It is now of interest to see if circular polarisation can be detected in the Crab nebula in the configuration suggested by the Rees and Gunn toroidal field model. Optical measurements by Martin *et al* (*Mon Not R Astr Soc*, **159**, 191, 1972) showed the presence of a weak circular polarisation but it is believed that this is produced by dust in the interstellar medium lying between the Crab nebula and the Earth.

No similar effect is produced by dust at radio wavelengths. An interesting new measurement of significantly higher sensitivity than made previously is now forthcoming. Weiler (this issue of *Nature*, page 24) reports the detection of circular polarisation in the radio emission from the Crab nebula at 1,415 MHz. Using the Westerbork Aperture Synthesis Radio telescope he finds a fractional polarisation of about 0.05% in four regions in the nebula. The circular polarisation is however of one sense only and does not show the change of sense in adjacent quadrants predicted on the toroidal model. Weiler concludes that the simplest magnetic field structure consistent with his observations is that of a relatively uniform field of 10^{-3} to 10^{-4} gauss with its line of sight component directed towards the Earth. On the face of it Weiler's detection is significantly above his measurement errors, although as he himself admits, independent confirmation would be useful at a lower frequency ($\lesssim 1$ GHz). If indeed this result is confirmed we are left with the need for a mechanism to maintain and amplify the magnetic field of the Crab nebula.

Models of nitrogen fixation

from A J Thomson

THE ability of primitive bacteria and some blue-green algae to reduce atmospheric nitrogen to ammonia at ambient temperature and pressure has long been a provocation to inorganic chemists. Until the mid-1960s, the only other known reaction of nitrogen at room temperature was the formation of metal nitrides either on clean metal surfaces or by lithium and alkaline earth metals. Considerable progress has been made in the isolation and analysis of the enzyme, nitrogenase, responsible for biological fixation (Hardy, Burns and Parshall, *Adv Chem Ser*, **100**, 219, 1971). High purity preparations are now available from three genera of bacteria, including *Azotobacter vinelandii* and *Clostridium pasteurianum*. The enzyme, which requires an electron source such as ferredoxin or sodium dithionite, can be separated into two proteins, a high molecular weight component containing one or, less probably, two atoms of molybdenum and about 15 atoms of iron per molybdenum atom. The smaller protein contains two atoms of iron and two ions of 'labile' sulphide. Little is known about the environment of the metal atoms, although the high sulphur content of the protein

has led to the suggestion that sulphur is a ligand. There is no direct evidence that the metal atoms are directly involved in binding nitrogen during fixation.

The discovery by Allen and Senoff (*Chem Commun*, 221, 1965) of the first metal dinitrogen complex, $[\text{Ru}(\text{NH}_3)_5(\text{N}_2)]^{2+}$, during the attempted synthesis of $[\text{Ru}(\text{NH}_3)_6]^{2+}$ by the reaction of hydrazine with ruthenium trichloride trihydrate in water showed that N_2 could be made to ligand to metals. This was an important step since it had been a puzzling feature of inorganic chemistry that, whereas so many carbon monoxide complexes of low-valent metals were known, the isoelectronic N_2 molecule was apparently unable to bind. Since this observation some fifty complexes of dinitrogen have been reported (Sellman, *Angew Chem Int Edit*, **13**, 639, 1974). It is now a tacit assumption of all model studies that a metal ion, most probably molybdenum, is responsible for binding dinitrogen in nitrogenase.

The primary process of fixation, the trapping of the nitrogen molecule at ambient partial pressure, has now been successfully modelled by several groups using well defined compounds. A group

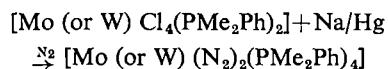
A hundred years ago



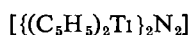
A RARE phenomenon, says the *Malta Times*, occurred in the forenoon of Monday, the 21st ult. During a strong wind from the south-west, which had prevailed for two days previously, the sea suddenly rose several feet and flooded the moles and roads surrounding the harbours, causing four or five steamers, moored between the Custom House and Calcara Rise, to snap their stern hawsers like packthreads, and carrying away boats that were hauled ashore in the French and other creeks. The sea then receded as suddenly as it rose, leaving portions of the bottom of the harbour exposed, upon which men and boys might be seen collecting fish and other marine animals that had been left aground by the retiring water. Shortly afterwards the sea resumed its ordinary level. Similar phenomena have been noticed occasionally during the course of many years.

From *Nature*, 11, 196, January 7, 1875

from the Agricultural Research Council Unit of Nitrogen Fixation at the University of Sussex were first to devise a reaction based, appropriately, on a molybdenum complex (Bell, Chatt and Leigh, *Chem Commun*, 482, 1970)

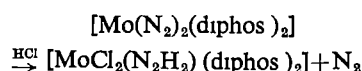


Subsequently, both Soviet and American teams succeeded in preparing



from gaseous nitrogen under mild conditions (Yu *et al.*, *Chem Commun*, 1178, 1972; Bercaw, Marvich, Bell and Brintzinger, *J Am Chem Soc*, 94, 1219, 1972; van Tamelen, Cretney, Klaentschi and Miller, *Chem Commun*, 481, 1972)

The second step required in the fixation process, the reduction of coordinated nitrogen to ammonia, has proved exceedingly difficult to achieve in any of the characterised complexes. Most attempts at reduction led to displacement of nitrogen. For example, solvolysis of $[(\text{C}_5\text{H}_5)_2\text{Ti}]_2\text{N}_2$ with methanolic HCl yields mainly nitrogen although ammonia and hydrazine appear as minor products. The first step in a well characterised partial reduction was achieved by Chatt, Heath and Richards in a reaction of their molybdenum (or tungsten) bis-dinitrogen complex with concentrated HCl (*Chem Commun*, 1010, 1972)



Now the persistence of the Sussex team has been rewarded. A report appearing in this issue of *Nature* (page 39) shows the room temperature reduction in a methanol/sulphuric acid solution of *cis* or *trans*- $[\text{Mo (or W)} (\text{N}_2)_2(\text{PR}_3)_4]$ to two molecules of ammonia and one of nitrogen. The yields of ammonia go as high as 90% per nitrogen molecule reduced for a *cis* tungsten complex. Recently an interesting parallel development occurred in the United States, with the demonstration of the partial reduction of dinitrogen bound to a zirconium analogue of the cyclopentadienyl complex (Manriquez and Bercaw, *J Am Chem Soc*, 96, 6229, 1974) $[\text{C}_5(\text{CH}_3)_5]_2\text{ZrCl}_2$ undergoes reversible binding of nitrogen slowly at atmospheric pressure in the presence of sodium amalgam to form a binuclear species $[\{\text{C}_5(\text{CH}_3)_5\}_2\text{Zr}(\text{N}_2)]$. At -80°C , this product undergoes reaction with 10 M excess HCl in toluene releasing two molecules of nitrogen gas and forming a white solid, $\text{N}_2\text{H}_4 \cdot 2\text{HCl}$.

There is an interesting contrast between the British and American reactions. The former seems to involve a metal ion oxidation state change of +6 to supply the reducing equivalents to produce two molecules of ammonia from one of nitrogen. In the American scheme, however, a dimeric metal complex supplies only four reducing equivalents to lower the oxidation state of N_2 to N_2H_4 . It has been thought extremely unlikely that the six reducing equivalents could be supplied by a single metal centre. It is a basic tenet of the theory of electron-transfer reactions, borne out by experiment, that probabilities of simultaneous transfers of two or more electrons are very low and indeed, there is now a general doctrine of compulsory one-electron steps in true electron transfer (as opposed to atom transfer) reactions. If reduction is brought about by a cluster of metal ions, a dimer for example, this difficulty is partially overcome. Thus the zirconium complex apparently involves only a two-electron transfer per metal atom. Such electron transfer processes are well known although taking place sequentially. The fact that nitrogenase contains a large number of metal ions lends some credence to the view that a multi-metal centre is responsible for reduction. A somewhat similar hypothesis persists for the terminal oxidases which supply four electrons for the reduction of oxygen to water. The presence of a number of metal redox sites in the enzymes again suggests a cooperative interaction between the centres to supply the necessary reducing equivalents without any one centre undergoing a large change in oxidation state.

For these reasons the claim by the Sussex group to have shown "that molecular nitrogen can be reduced at a single metal centre in a protic medium"

is important. It is clear that, according to the overall stoichiometry of the reaction, each metal ion supplies six reducing equivalents. But since no kinetic or mechanistic evidence is presented in this report, it is surely not yet possible to conclude that reduction has gone on at a single metal centre. The low oxidation states of molybdenum and tungsten are well known for a tendency to form clusters. A recent report (Cotton, Frenz and Webb, *J Am Chem Soc*, 95, 4431, 1973) describes the structures of two dimers of molybdenum, $[\text{Mo}_2(\text{SO}_4)_4]^{3-}$ and $[\text{Mo}_2(\text{SO}_4)_4]^{4-}$. Apparently sulphate ion, the medium used in the Sussex experiments, can bridge two molybdenum ions. Professor Chatt and his colleagues have themselves suggested that the difference between the susceptibility to solvolysis of the diphosphine and monophosphine complexes may reside in the lability towards substitution by sulphate ion of the latter ligand. In addition, they note the escape of one molecule of nitrogen on mixing the reagents. Thus, consideration should be given to the possibility that metal clusters are involved at some stage in multi-step reduction of nitrogen. Some mechanistic studies will clearly be necessary to resolve this problem. Although one may start a reaction with a "well-defined complex" and be able to write down the overall stoichiometry not all the intermediates are thereby defined. Undoubtedly these latest two reactions are going to provide useful models for exploration of the mechanisms and the intermediates by which multi-step reductions can occur.

Future for thin films

from A G Holmes-Stedle

THE table shown on page 9 was composed to stimulate discussion on the limitations and requirements for electronically active thin films when used for making solid-state devices. At present, almost all such devices are dependent on the use of major circuit elements made from single-crystal ingots (for example, most thin-film hybrid circuits contain a silicon transistor chip). The crystal must be grown with immense care, then sliced, polished, diffused and diced. More than 50 processing steps may thus be involved before a minute 'chip' with the required electronic functions is produced. The chip then has to be attached to a header and contacted by thin wires. Techniques are now available to produce many of the same electronic functions in the same volume of material using only thin layers which are deposited on an inert surface and then etched into complex patterns to form minute active circuit elements (see, for example, Maissel, *Handbook of Thin Film Technology*, McGraw-Hill, 1972).

Table 1 Twenty-two electronic devices which could be made entirely of thin films

A Most active material is semiconductor		B Most active material is dielectric
Bipolar transistor	Magnetoresistor	Variable-threshold transistor
MIS transistor*	Photoconductive photosensor	Dielectric filamentary switch
Schottky-barrier transistor	Mechanical transducer	Pyroelectric detector
Rectifier	Chalcogenide switch	Light-beam deflector
Junction photosensor	Chalcogenide memory	Light-beam modulator
MIS electroluminescent diode*	Chalcogenide triode	Storage photosensor
Solar cell	Photostructural switch	
Charge-coupled device	Cold-cathode electron emitter	

*MIS: Metal-insulator-semiconductor

The main technological skill which is still lacking is the ability to deposit films of the required crystal perfection for the performance of certain crucial electronic functions. In conventional single-crystal electronics, effects such as minority-carrier transport, avalanche multiplication, tunnelling, acoustic surface-wave transport are commonly employed to amplify or modify the electrical signal entering the device. If these processes are depressed by the various forms of disorder and defect state found in the thin-film form, then this device principle cannot be embodied in a structure made entirely of thin films. For example, surface acoustic waves (SAW) cannot be transmitted along an evaporated silica film because the film is glassy and hence is not piezoelectric. The SAW signal-processing device therefore does not appear on the table, though it would merit a place if current attempts to make oriented piezoelectric thin films of zinc oxide to serve the same purpose as the conventional quartz chip are successful.

Grown or deposited films may be completely amorphous (see, for example Le Comber and Mort, *Electronic and Structural Properties of Amorphous Semiconductors*; Academic Press, 1973) or may contain very large numbers of grain boundaries, dislocations or point defects. Whether a device should be fabricated by thin-film or single-crystal technology usually depends on the feasibility of controlling the disorder and defect content of the thin film to the required degree and the profitability of doing this on a mass-production scale. That the stakes are high is illustrated by Westinghouse's recent announcement of a flat all-thin-film display panel, measuring about 11 inches diagonally (released at the Institution of Electrical and Electronic Engineers International Electronic Devices Meeting, Washington, 20 October). This device ("The World's Largest Integrated Circuit") is a laboratory product, paid for by the military. Yet a form of it could appear in everyone's living room within a few years, displacing the bulky eye of the television cathode ray tube.

Other concerns are working on similarly attractive, "all-thin-film" possibilities such as the pocket television

camera, the pocket optical computer and the optical waveguide to replace electrical wiring. Some items in the table are well developed commercial devices which are now normally fabricated from ingots; others are still in developmental or conceptual stages. Some further details on the criteria used in making this list may be found in my paper in *Rep. Prog. Phys.* (36, 699; 1974), which deals with the special case of amorphous materials but employs a suitable analytical approach for discussing thin films in general. Note that it is not only semiconductors which must be considered: increasing use is now being made of electronically active dielectrics. Though the single-crystal approach will never be completely displaced, it seems certain that thin-film techniques for electronically active materials have an important future and active discussion between physicists and technologists can only serve to promote the best use of those techniques.

Volcanic tephra on Crete

from Peter J. Smith

EARTH scientists or archaeologists can sometimes obtain information of mutual interest about the past. For example Ninkovich and Heezen (*Colston Res. Soc. Papers*, 17, 413; 1965) concluded from their studies of Mediterranean deep sea cores that tephra from violent volcanic eruptions must have fallen on Crete at least twice in prehistoric times. The older eruption took place more than 25,000 years ago and scattered material throughout the whole island. In the more recent fall, thought to be related to the eruption of Santorini (Thera) about 3,500 years ago, tephra (the so-called Minoan tephra) apparently covered the eastern half of Crete to an average depth of about 10 cm.

At first sight, the new volcanological evidence relating to the more recent eruption seemed to support Marinatos's (*Antiquity*, 13, 425; 1939) earlier hypothesis that the eruption of Thera led directly to the sudden collapse of the Minoan civilisation. But the picture became cloudier when Olausson (*Opera Botanica*, 30, 29; 1971), who also studied deep sea cores, claimed that the

younger tephra identified by Ninkovich and Heezen really comprised three or four layers of different ages, the youngest being not less than 5,000 years old. There is also a problem at the field level in that many archaeologists have found no volcanic ash on Crete at all, and thus remain unconvinced that tephra from the Thera eruption even reached the island.

Moreover, the general archaeological background is not as clear as it might be, for whereas the general destruction on Crete apparently occurred towards the end of the Late Minoan IB stage in about 1450 BC, or 1470 BC at the very earliest, the ruins on Thera itself have so far yielded only one sample of pottery typical of that stage. This strongly implies that there was a gap of several tens of years between the destruction of Thera and that of Crete. But geologists have generally ruled out the idea of two violent paroxysms, pointing out that upheavals which could have produced the pumice observed in the area would hardly have been able to occur within thousands of years of each other. So if there was only one catastrophic eruption, did it destroy Thera (in which case it could not have been the direct cause of the destruction of Crete tens of years later) or did it destroy Crete (in which case archaeologists need to discover why Thera was abandoned tens of years before the eruption)?

A more thorough search for, and study of, Cretan tephra was clearly necessary and has now been made by Vitaliano and Vitaliano (*Am. J. Archaeol.*, 78, 19; 1974). But, first, what are the possible sources of tephra on Crete? There are five Aegean volcanoes with a late Quaternary history of violent explosive eruptions; but three (Nisyros, Yali and Kos) lie to the east-north-east of Crete and are thus considered unlikely sources because the high-altitude dust-transmitting winds in the region are predominantly from the west. This leaves only Melos and Santorini (Thera) to the north. Volcanic material from particularly violent eruptions can however travel very long distances. Some Cretan tephra could therefore come from as far away as Italy; and the 5,000 year old eruption mentioned by Ninkovich and Heezen is now thought to have been Italian.

The question that Vitaliano and Vitaliano set out to answer is thus as follows: can Thera tephra actually be found on Crete, identified as coming from the Minoan eruption of 3,500 years ago, and related to Minoan archaeological levels?

The question became one of the refractive index of glass. On Santorini the tephra overlying the Thera ruins largely comprises volcanic glass with a

refractive index of 1.509. By contrast, the glass from a previous Thera eruption 16,000–17,000 years ago has a refractive index of 1.514, that from Nisyros (though similar chemically to that from Santorini) has an index of 1.502, that from Melos has an index of 1.505 and that from Yali has an index of 1.494. Composition without optical properties is apparently not suitable for the purpose of determining the source of tephra, although a combination of composition and refractive index should be slightly better than index alone.

And tephra with glass of refractive index 1.509 is indeed found on Crete. Vitaliano and Vitaliano have now discovered the Minoan tephra in trace amounts in over 50% of soil samples collected along the eastern section of the north coast. Traces of the tephra are also found at various archaeological sites, though so far never in circumstances thought to represent undisturbed remnants of the original Minoan ash. A few particles of the 25,000-year-old Italian glass (index 1.52) were also found, but none from the 16,000-year-old glass of Thera.

Unfortunately, the quantities, locations and arrangements (with respect to archaeological artefacts) of the Minoan tephra are as yet insufficient to enable firm conclusions to be drawn about the relationship between the eruption and the destruction of Thera and Crete. But having proved the potential of their method, Vitaliano and Vitaliano tentatively conclude that "little or no time elapsed between the abandonment of Thera and the fall of tephra on eastern Crete". Thus the preliminary volcanological evidence suggests that the general destruction of Crete was not a direct result of the Santorini eruption.

The mating game

from our *Animal Ecology Correspondent*

THERE is no doubt that male and female animals play the mating game according to somewhat different rules. Males are usually much more showily dressed than females and are given to attention-seeking displays. Often these take place when there are no females in sight and so cannot be regarded as being for the benefit of the opposite sex. Darwin in 1871 (*Descent of Man and Selection in Relation to Sex*; Murray, London) stated that males competed among themselves for mates while females did not compete but exercised some choice in mate selection. In a thesis which received wide publicity in 1962, Wynne-Edwards stated that males displayed amongst themselves to assess their own density and decide which of them should breed and which should not (*Animal Dispersion in Relation to Social Behaviour*; Oliver

and Boyd, Edinburgh). The decisions are reached through competition for such prizes as a territory or a high rank in the social hierarchy. According to this thesis the fertilisation of ova is a secondary male role; the primary role is to ensure that the population is not allowed to exceed an optimum density above which habitat resources would be overstrained.

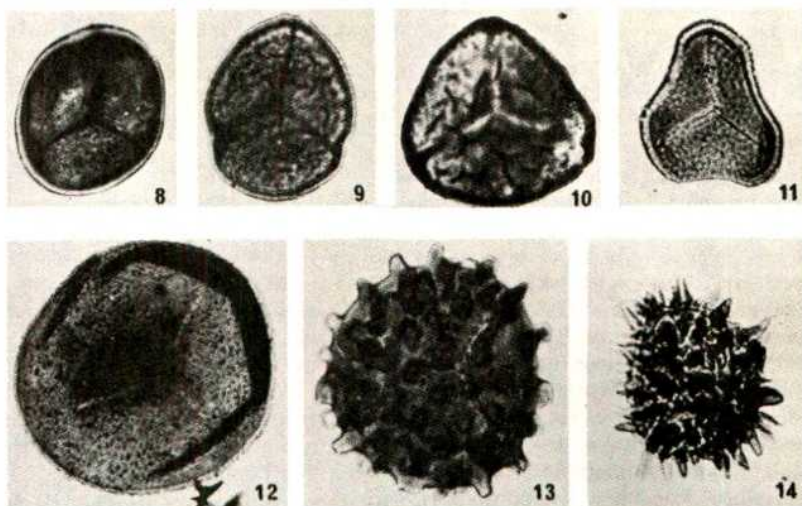
The late David Lack held firmly to the opinion that populations were kept below a level at which environmental damage would result by the action of predators or insect parasites (*Population Studies of Birds*; Clarendon Press, Oxford, 1966). In other words the size of the population is determined from without. Like Wynne-Edwards's, Lack's arguments are an extension of Nicholson's density-dependent control hypothesis (*J. anim. Ecol.*, 2, 132; 1933) inasmuch as there is a direct relationship between the severity of the controlling influence and the density of the population to be controlled. The displays which males put on apparently for other males, termed 'epideictic' by Wynne-Edwards, are seen by Lack to have nothing to do with a census but rather to have a sexual function in attracting females to males. In an early paper on black grouse (*Br. Birds*, 32, 290; 1939) he postulated that males displaying in groups attracted more hens than those displaying solitarily. He is thus critical of Wynne-Edwards's views that leks, traditional places for epideictic or nuptial displays, exist so that males can regulate the numbers of fe-

males fecundated and hence the population size.

Some long term studies of vertebrate leks are among the few studies of social behaviour in which observers have had the stamina to collect enough material to begin to assess these conflicting views.

The greater prairie chicken (*Tympanuchus cupido*) is a member of the grouse family which forms distinct leks. Robel and others (*Anim. Behav.*, 14, 328; 1966; *J. Wildl. Mgmt.*, 34, 306; 1970; *Auk*, 91, 75; 1970) have drawn attention to the fact that all matings take place at the lek. Between 1964 and 1969 there were few social upheavals at the chosen study leks and as a result 84% of the observed copulations were performed by dominant cocks. These also had the largest territories. This figure compares with 76% reported by Lumsden for dominant male sharptailed grouse *Res. rep. Ontario Dept Lands and Forests* No. 66, 1965 and 74% for dominant male sage grouse (Scott, *Auk*, 59, 477; 1942). During this long period of stability about 40 hens visited the lek each season of which, on average, 32 were mated. In 1970 three, and in 1971 two dominant cocks were removed from the lek and with them went the stability of the lek. The number of social interactions observed each morning, formerly running at 23, rose sharply to 109 and the number of females mated fell to three and two. Social unrest amongst the males clearly reduced the number of young produced by limiting

Miospore sequence from Scotland



Dinantian miospores from the Spilmersford Borehole in East Lothian, Scotland. The description of 45 specimens examined in a recently completed survey provides one of the first palynological reference sequences for the Lower Carboniferous in Scotland. 8, *Retusotriletes incohatus* Sullivan; 9 and 10, *Pulvinispora scolecophora* sp. nov.; 11, *Waltzispora planiangularata* Sullivan; 12, *Cyclogranisporites palaeophytus* sp. nov.; 13, *Raistrickia nigra* Love; 14, *Acoanthotriletes socraticus* sp. nov. In each case the magnification is 500X. Taken from the *Bulletin of the Geological Survey of Great Britain*, No. 45. (Her Majesty's Stationery Office, London, 1974.)

the number of females impregnated.

Elephant seals (*Mirounga angustirostris*) breed communally on selected small islands. Usually these are greatly overcrowded while other islands remain unburdened. In a six year study on Año Nuevo Island, California, Le Boeuf has focused attention on the small number of dominant males, usually fewer than one third of all resident males, which are responsible for the majority of copulations (*Am. Zool.* **14**, 163; 1974). Copulation frequency is directly related to success in competition between males for hierarchical accolade with alpha bulls being responsible for up to 49% of all matings. The Año Nuevo population is increasing, presumably due to subtle habitat changes, and with this increase is a decrease in the alphas' share of copulations. But there does seem to be some density dependent regulation in force. Le Boeuf in 1972 showed that a major source of pup mortality, up to 43% of all pups born, was the fighting of adult males (*Behaviour*, **41**, 1). Death to the pups was the result of being trampled upon by the bulls which weigh up to three tons. Crude though this mechanism may be, it is certainly effective. Social unrest among the males reduces the number of young produced by increasing infant mortality.

Some of the consequences of lek life in the Uganda kob antelope (*Adenota kob*) were reported in these columns a few weeks ago (**252**, 345; 1974). More than 15 years of study of the population in the Torro game reserve has revealed that there is a strong attachment to the home lek and adults of both sexes traditionally return home to breed (Buechner and Roth *Am Zool.*, **14**, 145; 1974). Only dominant males hold mating territories within the leks and, since clandestine matings are rare, they alone mate with females. There seems to be some limit to the number of territories in each lek, but what controls this is not known. Since rinderpest exterminated European cattle in this area kob populations have been increasing and a few additional leks are being formed. Population increase does not appear to be achieved by the establishment of additional territories within leks, but by the creation of additional leks.

Amid a welter of fascinating detail, these three studies reveal a couple of fundamental principles. First, they point out that Darwin's observations, *sensu stricto*, are incorrect because males fight not for females directly but for a conventional reward which itself confers the right to mate. Second, they show that social dominance is the key to conventional reward and hence to frequency of copulation. Additionally Robel's studies show that social stability between males is a major factor governing the number of females fecundated

and in Le Boeuf's study social interaction strongly influences infant survival.

Neither Lack's nor Wynne-Edwards's disquisitions can be proved or disproved by these studies although there is strong *prima facie* evidence that the behaviour of males has a regulatory function on the number of new individuals brought into the population. What has not been shown is whether leks serve primarily to census populations and their resources or to provide an attractive meeting, and mating, place. More long term studies may help to reveal the complications thrown in by such factors as overall population increase and so lead towards a correct interpretation of the function of leks. They may also, as a sideline help to indicate the area in which the regulation of human populations went awry.

Adapting to a chilly future

from Peter D. Moore

IF "The Threat of Ice" (see *Nature*, **252**, 216; 1974) is a real one, it may be salutary to learn how a variety of organisms adapt to cold. The adaptations of plants, which cannot migrate, must be physiological; they are also seasonal, in all but the most severe arctic and alpine climates. It has long been realised that the ability of many plants to tolerate cold varies with their recent temperature experiences (Levitt, *Handb. Pflanzenphysiol.*, **II**, 632; 1956); this knowledge has been used in the horticultural practice of frost hardening. Riedmüller-Schölm (*Flora, Jena*, **163**, 230; 1974) has recently examined the low temperature resistance of many Alaskan plants at monthly intervals

Another function for juvenile hormone

from our Insect Physiology correspondent

ONE attraction of the hormonal system of insects as a medium for the study of endocrinology has lain in the apparent simplicity of the range of hormones involved. As knowledge grows complexity increases. The diversity and subtlety of the effects of the juvenile hormone seem to militate against the formulation of any unitary theory of the control of metamorphosis. But a new factor has come to light in the course of studies by Nijhout and Williams (*J. exp. Biol.*, **61**, 481 and 493) on the tobacco hornworm *Manduca*. Throughout the early larval stages of *Manduca* there seems to be a continuing cycle of secretion of the brain hormone (the prothoracic-tropic hormone: PTTH) which induces the discharge of ecdysone by the prothoracic gland and of juvenile hormone from the corpus allatum, which ensures the maintenance of larval characters. But in the fifth or final larval stage the scene changes: feeding during this stage is more prolonged and when moulting does take place the larva suffers metamorphosis to the pupa.

One thing can be predicted from existing knowledge: the brain must not release PTTH while a large amount of juvenile hormone is circulating, or another larval stage will be produced. What is peculiar about the final larval stage, according to the results of Nijhout and Williams, is that the high titre of juvenile hormone inhibits release of PTTH by the brain. But when the larva reaches a certain weight (about 5 g) the corpora allata ceases

to secrete the juvenile hormone. Within about 24 h the juvenile hormone is cleared from the haemolymph and this results in the brain being rendered competent to release PTTH during the ensuing photoperiod. Growth continues for several days to give a final weight of about 8 g. The gut contents are then discharged and the behavioural and developmental changes that lead to pupation are set in train.

These observations provide an informative description of the hormonal changes during the last larval stage in Lepidoptera; and they illustrate a new function for the juvenile hormone. The failure of the normal arrest in juvenile hormone secretion is apparently responsible for the developmental arrest or diapause in the mature larvae of some Lepidoptera. Fukaya and Mitsuhashi and more recently Chippendale and Yin have stressed the importance of juvenile hormone in inducing larval dormancy. The onset and persistence of diapause in mature larvae can now be accounted for by a functional failure of the normal mechanism which inactivates the corpora allata of non-diapausing larvae.

The turning off of the corpus allatum secretion not only permits the morphological change of metamorphosis; it now seems to set in motion in caterpillars the endocrine events that lead to the moult itself. But what turns off the corpus allatum? Some environmental stimulus? Some proprioceptive stimulus? Or the passage of time?

through the course of a year and has found, rather predictably, that the highest degree of tolerance occurs in midwinter, though many species have a secondary peak of resistance at the end of winter, associated with the melting of snow cover. During these frost-hardy periods most plants examined (including angiosperms, gymnosperms, pteridophytes, bryophytes and lichens) were able to withstand a temperature of -80°C , in some cases for several days. Since the lowest observed temperature in the field during this time was -52°C the adaptations seem to be quite adequate for survival.

Perhaps surprisingly, maximum heat resistance was found to coincide with maximum frost tolerance in midwinter. For mosses these maxima ranged between 48 and 60°C , for vascular plants from 56 to 72°C and for lichens between 66 and 74°C . These figures exceed the published heat tolerance limits for plants of any climate, including tropical ones.

These data are all the more remarkable when one considers that many arctic and alpine plant species seem to be limited in their geographical distributions by high summer temperature (see Conolly and Dahl in *Studies in the Vegetational History of the British Isles*, edit. by Walker and West, page 159; Cambridge University Press, 1970). There are two points, however, which may help to explain the seeming anomaly. In the first place, high temperature resistance is very much less marked in summer than in winter; in the second place, temperature tolerance does not necessarily ensure that a plant would survive long periods at fairly high temperatures. Billings and Mooney (*Biol. Rev.*, **43**, 481; 1968) concluded that arctic/alpine plants have considerably higher respiration rates than do most temperate species. It could prove difficult for such plants to maintain a positive carbon balance at prolonged high temperatures.

The winter peak in high temperature tolerance must be regarded as a side-effect of the very physiological changes which provide low-temperature resistance. This has been observed previously, as has the drought resistance of such frost-hardy plants. The mechanism underlying these changes has recently been sought in cell membranes (see, for example, Raison, *Symp. Soc. exp. Biol.*, **27**, 485; 1973) particularly in mitochondria from fruit and vegetables. Wilson and Crawford (*J. exp. Bot.*, **25**, 121; 1974 and *New Phytol.*, **73**, 805; 1974) have now examined the level of unsaturated fatty acids in leaves during the development of low-temperature resistance. Using *Gossypium hirsutum* and *Phaseolus vulgaris* they demonstrated that the degree of unsaturation of the fatty acids associated with the

phospholipids (in the membranes of leaves) increased during the process of frost hardening. This was due in particular to increases in the percentage of linolenic acid in the phospholipids. It is suggested that the significance of this change is to lower the temperature at which a phase change occurs within these membranes from a liquid crystalline phase to a solid gel phase. A similar mechanism has also been suggested for poikilothermic animals. Such a membrane modification may have both a structural and a metabolic role by making membranes less susceptible to damage and allowing greater activity in certain enzymes.

Growth at 25°C also resulted in a higher degree of unsaturation in the phospholipid fatty acids, suggesting that this mechanism is also associated with high temperature tolerance. But tropical plants may have lost the capacity to increase the degree of fatty acid unsaturation in their phospholipids over short periods of time, thus rendering them sensitive to very low temperatures. As the next ice advance approaches, let us hope that we all have time to adapt.

Agglutination, proteolysis and cell division

from Robert Shields

WORKERS studying the control of cell proliferation have long been intrigued by the greatly decreased rate of DNA synthesis by normal cells in tissue culture when access to serum is restricted. When normal cultures reach their maximum (saturation) density the vast majority of the cells are in the G_1 phase of the cell cycle. Such cultures will undergo limited proliferation if the cells are treated with low doses of proteases or are given fresh serum. Under the same conditions tumour cells are not so subject to serum limitation; they will grow to far higher densities and are distributed throughout the cell cycle.

As tumour cells are more easily agglutinated by multivalent lectins such as concanavalin A (Con A) and confluent normal cells show transient increases in agglutinability when growth is stimulated with proteases, it was thought that exposure of lectin binding sites might regulate cell division. Experiments by Burger (*Nature*, **228**, 512; 1970) on transformed 3T3 cells with a preparation of trypsinised Con A (which was thought to be monovalent and so non-agglutinating) seemed to indicate that normal morphology and low saturation densities could be restored to the tumour cells by covering the Con A receptors on the cell surface.

A recent paper by Trowbridge and Hilborn (*Nature*, **250**, 304; 1974) has questioned these results; using the better characterised succinyl Con A (which is divalent) it was shown that covering the agglutination sites neither restored a low saturation density to transformed cells nor reduced DNA synthesis nor arrested cells in G_1 . These authors suggest that the restored saturation density seen by Burger may have been the result of cell death caused by the trypsinised lectin.

It is now realised that exposure of agglutination sites is similar in normal and transformed cells and that agglutinability requires rearrangement of these sites, which occurs more readily in transformed cells. It remained possible, however, that agglutinability *per se* was a sufficient condition for cell division until experiments with pronase demonstrated that density-restricted 3T3 cells could be made maximally agglutinable without cell division occurring (Glynn, Thrash and Cunningham, *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2676; 1973).

A proteolytic activity on the transformed cell membrane seemed an attractive hypothesis in view of the similarities between transformed cells and protease-treated normal cells. The protease was thought to be responsible both for the high agglutinability and for an autocatalytic stimulation of division. Transformed cells were indeed found to have a greater proteolytic activity than their normal counterparts and moreover to have a proteolytic activity that converted plasminogen (present in the serum of the culture medium) to the protease plasmin. Reich and his co-workers (*J. exp. Med.*, **138**, 1056; 1973) showed that several of the characteristics of transformed cells such as altered morphology and the ability to grow in agar seemed to depend on plasmin activity. If these protease activities were really important in cell transformation the corollary was that inhibition of these proteases should cause phenotypic reversion of the transformed cells.

Once again early experiments looked promising. The growth of transformed cells was reduced and a lowered saturation was restored with protease inhibitors (Schnebli and Burger, *Proc. natn. Acad. Sci. U.S.A.*, **69**, 3825; 1972). With some protease inhibitors the cells showed a more normal morphology and had reduced agglutinability. But by this stage of the game it was becoming apparent that a lowered saturation density and morphological changes were poor evidence for restoration of density restriction of growth. A flurry of papers showed that inhibitors of plasminogen activation did not restore a low saturation density to transformed cells (Chou, Black and Roblin, *Nature*,

250, 739; 1974), and other protease inhibitors had no selective effect on tumour cell growth (McIlhinney and Hogan, *Biochem. biophys. Res. Commun.*, **60**, 348; 1974). Furthermore those that did arrested cells in the G₂ phase of the cell cycle (Collard and Smets, *Expl Cell Res.*, **86**, 75; 1974) and the cells continued to make DNA at their saturation densities (Schnebli and Haemmerli, *Nature*, **248**, 150; 1974). But these results only show that normal cell properties cannot be restored by certain protease inhibitors; they cannot rule out the possibility that some proteases are involved in maintaining the transformed state.

More antigens in hepatitis B

from Arie J. Zuckerman

AUSTRALIA ANTIGEN, now referred to as hepatitis B surface antigen in view of its close association with hepatitis B virus, was discovered as a lipoprotein which was immunologically distinct from normal low density lipoproteins. Examination of this antigen in the electron microscope revealed a remarkable morphological heterogeneity consisting of three principal virus-like particles (see Fig. 1). The main antigenic constituent is a small pleomorphic particle, 16–25 nm in diameter. A characteristic feature is the presence of tubular forms which vary greatly in length but with a constant diameter of 20 nm except for the bulbous swelling at one or both ends of the tubules. The third type of particle, the Dane particle, is also spheroidal measuring about 42 nm in diameter, with a 27 nm core, a 2 nm shell and an outer coat about 7 nm in thickness. There is now substantial evidence that the 42 nm particle is the human hepatitis B virus, the core being the nucleocapsid and the outer protein coat representing hepatitis B surface antigen. Immune electron microscopy and more recently various serological tests revealed the existence of two distinct antigen-antibody systems associated with the Dane particle; antibody to the core has a specificity entirely different from antibody against the outer protein coat. The morphological complexity of the particles associated with hepatitis B is matched by the complex antigenic reactivities of the outer coat. The antigenic constitution of the core is still under investigation.

The antigenic activity of the coat protein is associated with the small spherical particles, the tubular forms and the coat of the Dane particles. All these structures share a common group specific antigen (a) and the particles generally carry at least two sub-determinants: either d or y, which usually behave in a mutually exclusive

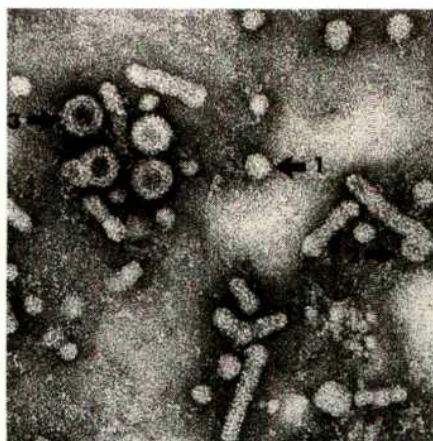


Fig. 1 Morphological appearance of hepatitis B antigen in serum showing three distinct entities: (1) Small pleomorphic spherical particles with a diameter of 16–25 nm. (2) Tubular forms. A terminal bulbous swelling is shown. (3) Double-shelled 42 nm Dane particles with a core and an outer coat. $\times 252,000$. (Electron micrograph reproduced with permission from A. J. Zuckerman *Hepatitis-Associated Antigen and Viruses*; North-Holland, 1972.)

manner although carried on the same antigen particle, and either w or r. There is evidence that the subtypes are the phenotypic expressions of distinct genotype variants of hepatitis B virus. Four principal phenotypes are currently recognised: adw, adr, ayw and ayr, but others are not precluded. Indeed complex permutations of these subdeterminants and new variants have been described, all apparently on the surface of the same physical particles. A remarkable geographical pattern of distribution of hepatitis B subtypes has emerged with four global zones, where there is an excess of one subtype, and regions where a mixture of subtypes is common (*Nature*, **247**, 2; 1974). These subtypes provide valuable epidemiological markers and offer a method for distinguishing one of several sources of infection. The surface antigenic reactivities do not seem to be associated with particular clinical forms of liver disease.

The above summary serves as an introduction to another antigen-antibody complex which is associated with molecules distinct from particles of hepatitis B antigen. Magnus and Espmark (*Acta path. microbiol. Scand.*, **B80**, 335; 1972) described a new distinct precipitating antigen, which they termed e, in sera containing hepatitis B surface antigen. This antigen differed markedly from the previously described determinants of the surface antigen. Paradoxically, antibody against e was found in serum specimens from healthy carriers of the surface antigen. The e antigen seemed to be somehow intimately associated with the pathogenesis of liver damage. Recently, Nielsen and col-

leagues (*Lancet*, **ii**, 913; 1974) reported their interesting observations on the e determinant. The e antigen was found by the simple immunodiffusion technique to be significantly more common in patients with chronic liver disease (chronic hepatitis and cirrhosis) with persistent hepatitis B antigenaemia than in patients with acute viral hepatitis. Furthermore, the e antigen seemed to be a valuable and important prognostic marker since progression to chronic liver disease was recorded by serial liver biopsies in 11 out of 19 consecutive patients with surface antigen-positive acute hepatitis associated with the e antigen. The clinical significance of the e antigen was supported by differences in the clinical, biochemical and histological findings between the patients with the e antigen and those without this antigen during the initial phase of viral hepatitis.

The exact nature of the e antigen is uncertain; it might be a host antigen produced by virus-infected liver cells or it might be related to another antigenic constituent of the infecting virus, perhaps the core of the Dane particle. Preliminary results from Nielsen's laboratory showed a large number of Dane particles in serum samples containing the e antigen, whereas e antibodies have previously been found in healthy carriers of the surface antigen in whom Dane particles had not been demonstrated. Though the precise relationship of e to hepatitis B virus is yet to be established, it is evident that the antigenic complexity of this unique infectious agent will continue to unravel.

Position effects in gene inactivation

from Benjamin Lewin

GENES translocated to positions close to heterochromatin seem in many species to become inactivated. Among the earlier observations were those made in *Drosophila* where, for example, some of the facets of the eye are white instead of red in heterozygotes in which the dominant gene has been transferred to a site adjacent to heterochromatin (see Lewis, *Adv. Genet.*, **3**, 75–115; 1950). It has since become apparent that this position effect variegation takes place in *Drosophila* when a wild type allele is placed in *cis* alignment with heterochromatin, with a recessive allele carried on the normal homologue; the wild type allele is inactivated in some cells, thus causing the variegation. The probability that a locus will be subject to this effect depends upon its relationship to the site of translocation; the closer it lies to heterochromatin, the more likely it is to be inactivated. Inactivation is de-

terminated during larval embryogenesis.

Although similar situations arise in the mouse, the rearrangements that cause the variegation always involve X-autosome translocations; in female mammals, one of the two X chromosomes, chosen at random, is inactivated in each somatic cell at any early stage of development, and the position effect can thus be shown only in those cells in which the rearranged X chromosome is inactivated to form heterochromatin. X inactivation occurs when the number of cells in the embryo is small, probably about 60 (Ohno *et al.*, *Cell*, **1**, 175-191; 1974; for review see Lewin, *Nature*, **249**, 9-11; 1974). This generates clones of cells in which only either the maternal or the paternal X chromosome is active, giving rise to variegation in the adult for any X-linked genes for which it is heterozygous. Thus although in an earlier study Cattanaach, Wolfe and Lyon (*Genet. Res.*, **19**, 213-228; 1972) showed that the variegated coats of mice heterozygous for an X-autosome translocation display a pattern similar to that of chimaeric animals, the conclusion that the autosomal locus on the translocated X chromosome is inactivated in early development does not distinguish between random X inactivation and position effect variegation. An analysis which allows these phenomena to be resolved is now reported by Cattanaach (*Genet. Res.*, **23**, 291-306; 1974).

The heterozygotes used in this study possess an X chromosome, X^T , that carries part of autosome 7; an X chromosome, X^N , that is normal; and a normal set of autosomes. The insertion within X^T carries the wild type genes $+^o$ and $+^p$ for coat colour; the normal copies of autosome 7 carry the recessive alleles c (albino) or its alternative c^{ch} (chinchilla) and p (pink-eye). Expression of the autosomal recessive coat colour alleles occurs in cells in which the insertion within X^T has been inactivated; coat colour genes are expressed by the melanocytes, which reach their ultimate positions in the hair follicles by migrating laterally down the foetal body. Each clone of melanocytes gives rise to a band across one side of the mouse coat; the clarity of these bands was enhanced in these experiments by use of the gene s , recessive spotting, which appears to reduce intermingling between cells of adjacent bands. Three sets of experiments were performed with this system.

In animals that suffered random inactivation, expression of the p and c^{ch} genes was followed in the bands. Three types of band were observed: $+^p +^o$ (wild type colour); $p c^{ch}$ (white); and $+^p c^{ch}$ (brown). The $+^p +^o$ bands are derived from cells in which the translocation chromosome X^T was active;

the $p c^{ch}$ bands represent cells in which X^T was inactive and both the $+^p$ and $+^o$ alleles on its insertion were inactivated. This confirms the clonal nature of the lines descended from cells in which either X^N or X^T respectively were inactivated. The third band, $+^p c^{ch}$, however, is derived from cells in which X^T was inactivated; but although the inactivation extended to $+^o$ it did not include $+^p$, which remained active. Since the $+^o$ locus is closer to the material of the X chromosome than is the $+^p$ locus, this is in accord with earlier experiments that showed that inactivation spreads sequentially from the X chromosome into the inserted autosomal material. By obtaining variegation of one of the inserted genes relative to the other, these results demonstrate that position effect variegation in the mouse is clonal and must have occurred early in development, between the time of X inactivation and the colonisation by melanocytes.

The animals used in these experiments had the genotype Xce^b/Xce^b , where Xce is a locus on the X chromosome which can influence the usually random pattern of inactivation; the Xce^b allele (previously known as Xce^1) permits random inactivation. But the Xce^a allele (previously described as Xce^c) causes the X chromosome carrying it to be inactivated more frequently in Xce^a/Xce^b heterozygotes; and in experiments in which the X^T chromosome carried the Xce^a allele (its normal homologue carrying Xce^b), the frequency of $+^p c^{ch}$ bands was increased, for the number of cells with $+^p$ active and $+^o$ inactivated was increased.

In the second set of experiments, random X inactivation was suppressed by using an X chromosome which carried Searle's translocation; this X is never inactivated, so it is the genes carried by the other X chromosome, in these experiments X^T , that fail to be expressed. But mice which carry $+^o$ on X^T and carry c on both copies of autosome 7 may still display some variegation for the c phenotype. Thus although X^T is inactive, the $+^o$ gene on the inserted autosome segment can still be expressed, albeit at frequencies less than 5%. The pigmented areas are always small—much smaller than whole bands. When the autosomes of such mice carry p as well as c , extensive pigmentation occurs in the production of $+^p c^{ch}$ regions, in which $+^p$ must be active in the inactivated X^T chromosome, although its $+^o$ gene remains inactive. The $+^p c^{ch}$ regions may occupy whole bands; this suggests that only genetic events are implicated in their establishment, excluding alternative explanations such as cell selection. By extension, this argument implies that the small pigmented areas in the mice where only the c locus is the sub-

ject of variegation also must have a genetic origin; so these must be derived either by late determination or by a reversal of an earlier inactivation.

In the third series of experiments, animals whose variegation had been examined were kept for up to 18 months and scrutinised for changes in coat colour as ageing took place. It has been observed previously that in general c areas uniformly darken with age. This suggests that a reversal of inactivation takes place with time, leading to expression of the $+^o$ gene on X^T . When the $p c^{ch}$ areas of the mice used in Cattanaach's experiments were examined, he found that they first turn the brown colour characteristic of $+^p c^{ch}$ cells and then turn the black colour characteristic of $+^p +^o$ cells. This suggests that the inactivating influence along the autosome insertion within the X chromosome gradually retreats, causing sequential reactivation.

From these results, Cattanaach proposes an alternative to the previously proposed explanations of position effect variegation in the mouse. The variegation has usually been attributed to a sequential inactivation proceeding along the translocated autosomal region from the X chromosome. This might involve structural changes, or, as has been suggested for *Drosophila*, the addition of suppressor molecules to the inactivated regions. Given that reactivation is responsible for events occurring later in development, however, Cattanaach argues that perhaps only one mechanism is responsible for all variegation, irrespective of whether it takes place early or late. Thus after inactivation of the translocated autosome (possibly still to a limited extent), a progressive reactivation takes place with time from the point most distant from the site of translocation; this must vary in rate in different cells. If reactivation at any locus occurs at an early stage, an active gene will be clonally inherited; although the descendants of this cell all will possess this activity, subclones may be generated as progressive reactivation applies to other loci. This model has the advantage that the position effect can be explained by a single mechanism operating at all stages of development, in contrast with models where stability of the inactivated autosome region is implied and for which an alternative model must be invoked to explain reactivation. Since there is no suggestion that the reactivation applies to genes of the X chromosome itself, the model draws a clear distinction between the sex heterochromatin and the autosomal insertion, although no implications are made about the molecular mechanisms that may maintain the inactive X but progressively fail to inactivate the autosomal region.

articles

Unconformities in the sediments of the Indian Ocean

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Deep sea drilling in the Indian Ocean has revealed unconformities of oceanwide significance. The unconformities are of Oligocene, early Tertiary and late Cretaceous age and can be explained as a consequence of climatic events in Antarctica and subsequent variations in the circulation pattern of the whole Indian Ocean.

UNTIL recently it was believed that sedimentation in the deep oceans is essentially continuous. One of the more unexpected results of the Deep Sea Drilling Project (DSDP) has been the discovery of large gaps in the sedimentary record at many sites. Major hiatuses of regional significance have now been identified in the Atlantic, Caribbean, Pacific, Antarctic and Indian Oceans. Data from sites drilled on DSDP Legs 22-27 and part of 28 (Fig. 1, refs 1-7) show that in the Indian Ocean gaps in the sedimentary record have been encountered in a broad spectrum of terrigenous, pelagic, biogenic and volcanogenic sediments encompassing late Mesozoic and Cainozoic time. The hiatuses were encountered at more than half the 48 sites and are developed on a wide variety of topographic features over a broad range of depths. They seem to show temporal groupings centred in the Oligocene, early Tertiary, and late Cretaceous.

Here we use the term unconformity to refer to a significant gap (demonstrated or inferred) in the stratigraphic record (disconformity or paraconformity). Unconformities are considered to represent active erosion or complete non-deposition of sediment. At many sites undated intervals were encountered, barren of microfossils, usually with a lithology of detrital or pelagic clay accompanied by zeolites and other indicators of extremely slow sedimentation. These intervals, which may be considered dissolution facies, represent slow sediment accumulation beneath the carbonate compensation depth (CCD). They may or may not contain unconformities, but this cannot easily be resolved because of the lack of biostratigraphic control. There is indirect evidence that in many cases the undated intervals do encompass unconformities.

The descriptions of unconformities from the results of some of the earlier legs of the drilling programme have been somewhat equivocal because of the often large unsampled intervals in the cores. Fortunately, in the Indian Ocean programme,

continuous coring or instances of greater than 50% coring were frequent. So we have considerably more confidence in recognising unconformities and in determining their extent. The Oligocene unconformity can be recognised with certainty at 15 of the 33 sites sampling that time interval and there is good evidence for its presence at 11 further sites making a total of 26 out of 33 sites. Corresponding figures for the early Tertiary and late Cretaceous unconformities are 18, maybe 27, out of 30 sites, and 9, maybe 13, out of 13 sites, respectively. It is clear, therefore, that these unconformities are of oceanwide significance. We believe that they can be explained as the consequence of climatic events in Antarctica and subsequent variations in the circulation pattern of the whole Indian Ocean.

Stratigraphic record in the Indian Ocean

Western Indian Ocean. In Fig. 2 the stratigraphic intervals recorded at sites in the western Indian Ocean are shown projected into a north-south line (locations in Fig. 1). Unconformities and/or undated intervals are shown centred on the Oligocene and the early Tertiary (or latest Cretaceous). The crust in the western Indian Ocean is generally younger than the eastern basin, with most western sites being no older than latest late Cretaceous. Consequently, a separate late Cretaceous hiatus is recognisable at only a few sites. True unconformities were encountered at 11 sites. Undated intervals, generally represented by a dissolution facies of zeolitic clay, are equally common. In many cases, such as sites 248 and 250, the sediments in these intervals are thin relative to those above or below, implying that the undated intervals do contain true unconformities. Local unconformities are indicated since the late Miocene at Site 240 in the Somali Basin and in the Pliocene at Site 249 atop the Mozambique Plateau, as well as at sites in the north-western basins. The maximum duration of the Oligocene hiatus is from the late Cretaceous to the early or middle Miocene (Site 249), indicating that the physical causes of the disconformities and dissolution facies ceased operating in the early/middle Miocene but could have been initiated at any time prior to this back to the late Cretaceous. The time-span of the early Tertiary hiatus extends from at least the Maestrichtian (Site 248) to late Eocene (Site 239).

Generally it seems that the cause of these unconformities is pervasive throughout the water column since both shallow and deep sea floor have been affected.

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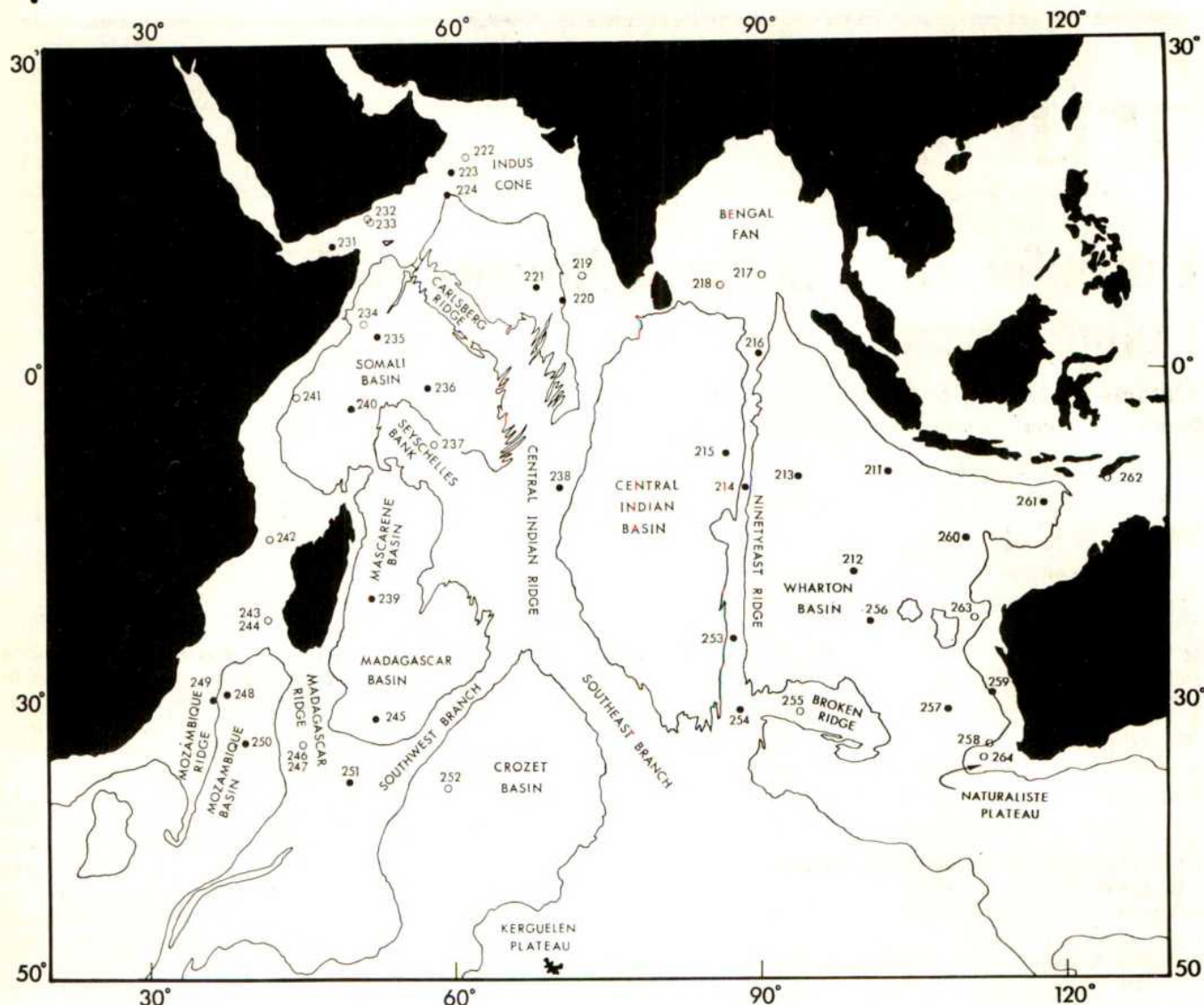


Fig. 1 Deep Sea Drilling Project sites in the Indian Ocean. ●, Sites which reached basement; ○, other sites. The principal physiographic features are defined by the 4,000 m contour.

Eastern Indian Ocean. Data from sites in the eastern Indian Ocean are interpreted to show hiatuses centred on the Oligocene, early Tertiary and/or late Cretaceous (Fig. 3). The upper limit of the Oligocene hiatus in Figure 3 is in the late Miocene or Pliocene (Sites 264, 258, 261, 215). The limits of the earlier hiatuses are not well defined but we interpret the upper limit of the early Tertiary hiatus to be in the middle Eocene (Site 216, 217) and the limit of the Cretaceous hiatus may be as young as late Palaeocene (Site 264).

Unconformities are again present in a variety of bathymetric features. They are found in the shallow sites on the Naturaliste Plateau, Ninetyeast Ridge and Broken Ridge and at deep sites such as 213, 215, 259 and 263. At the deeper sites, undated intervals—which may or may not include unconformities—are common.

Comparing Figs 2 and 3 we see that the hiatus centred on the Oligocene ends in the middle Miocene in the west and in the late Miocene/Pliocene in the east. On the Ninetyeast Ridge, the Oligocene hiatus ends in the late Oligocene, suggesting that if the same physical cause is responsible for both the shallow and deep hiatuses, it ceased functioning in the shallow regions first. The early Tertiary unconformity ends in the early Eocene in the west and middle Eocene in the east. The causes of both these unconformities seem to have ceased operating sooner in the west than in the east.

Regional extent of the Cainozoic hiatuses

The danger of regionalising these observed hiatuses cannot be overemphasised, considering our imperfect knowledge of the history of the local sedimentation regimes at each site. Nevertheless it is instructive to try.

In Fig. 4 we have plotted Oligocene and older DSDP sites from Legs 21 to 28 on a late Oligocene tectonic reconstruction. The extent of the Oligocene hiatus is indicated by the shaded regions. The hiatus is present at both shallow and deep sites whereas a complete Oligocene section (apparent) was recovered in the north-west Indian Ocean at shallower sites near the ancient ridge crest and nearer to the palaeoequator. Figure 4 shows that the western Pacific sites of DSDP Leg 21 exhibit the same hiatus. Kennett *et al.*⁸ described this hiatus as a disconformity found at various depths between 1,400 and 4,500 m. It seems to end in the middle Miocene, about the same time as in the western Indian Ocean. Kennett *et al.* pointed out that Oligocene unconformities are also found in southern Australia, New Guinea and parts of New Zealand⁹⁻¹¹. Carter and Landis¹² described the south Australian unconformity as a paraconformity below shelf limestones of early Miocene age. Oligocene hiatuses are not generally found in the central Pacific. The south central Pacific DSDP Leg 8 results show a hiatus near the late Eocene/early Oligocene but the hiatus itself is marked by calcareous ooze with very high ^{137}Cs concentrations.

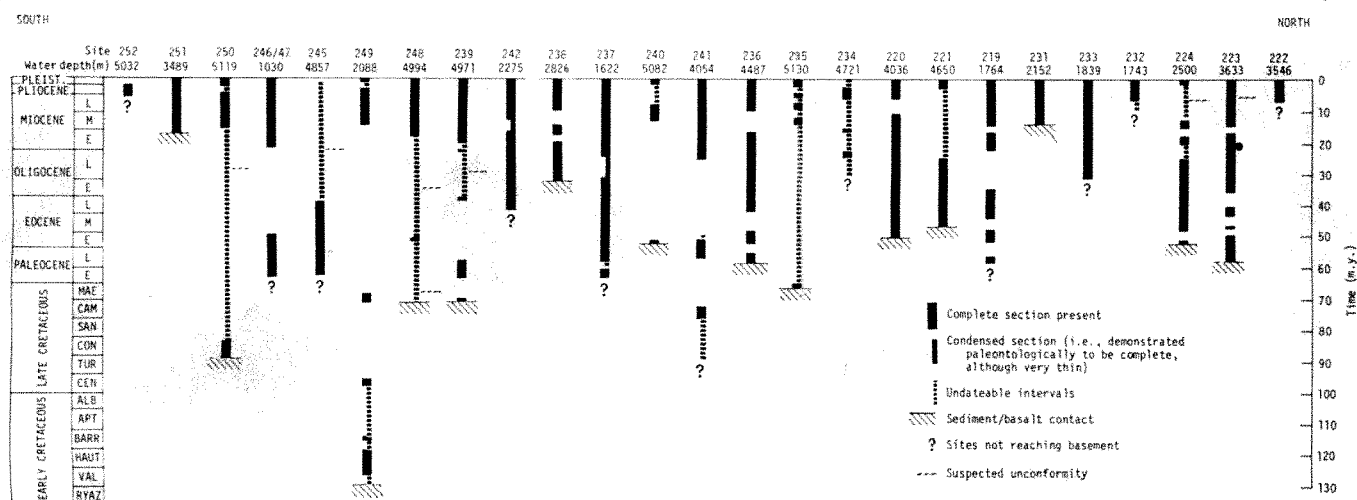


Fig. 2 Stratigraphic section sampled at sites in the western Indian Ocean. Sites 243 and 244 are omitted since no cores were recovered from these sites. Basement ages at Sites 248 and 249 were determined radiometrically⁴.

rates¹⁴. High calcareous sedimentation was also seen in the sediments sampled on DSDP Leg 3 in the South Atlantic¹⁵, where the Oligocene is represented by two chalk ooze formations. Clearly, erosion and/or non-deposition and dissolution in the Indian Ocean and south-west Pacific during the Oligocene was contemporaneous with high carbonate deposition in other oceans of the world.

In Fig. 5 we have plotted Indian Ocean and south-western Pacific DSDP sites on an early Eocene reconstruction. The early Tertiary hiatus is more difficult to regionalise because it is often obscured by the overlying Oligocene hiatus and at many sites the oldest sediments recovered were younger than Eocene. But certain patterns seem similar to those of the Oligocene data; these include uninterrupted sedimentation in the north-west, unconformities on shallow structures such as the Ninetyeast Ridge, and a well developed hiatus in southerly latitudes. The similarity in the regional patterns of the early Tertiary and Oligocene hiatuses implies that they may be due to similar causes.

The early Tertiary hiatus is documented in the central Pacific by DSDP Leg 17 results¹⁶. This hiatus is a disconformity which ends in the middle Eocene in both shallow (2,300 m, Site 171) and deep (5,500 m, Site 164) sites. The central Pacific hiatus ends at the same time as the early Tertiary hiatus on the Ninetyeast Ridge. Leg 21 results also show a hiatus ending in the middle Eocene in the southwest Pacific⁸ (Fig. 5). In the South Atlantic, the early Tertiary sequence is complete¹⁵.

Late Cretaceous sediments have not often been sampled by DSDP, but in the central Pacific some Leg 17 results indicate an unconformity ending in the Campanian as in the Indian Ocean.

It seems, therefore, that regional hiatuses in the Cretaceous and early Tertiary in the Indian Ocean are closely allied with contemporaneous ones in the central and south-west Pacific. The Oligocene hiatus in the Indian Ocean and south-west Pacific, however, occurred at the same time as high calcareous sedimentation in the central Pacific and South Atlantic.

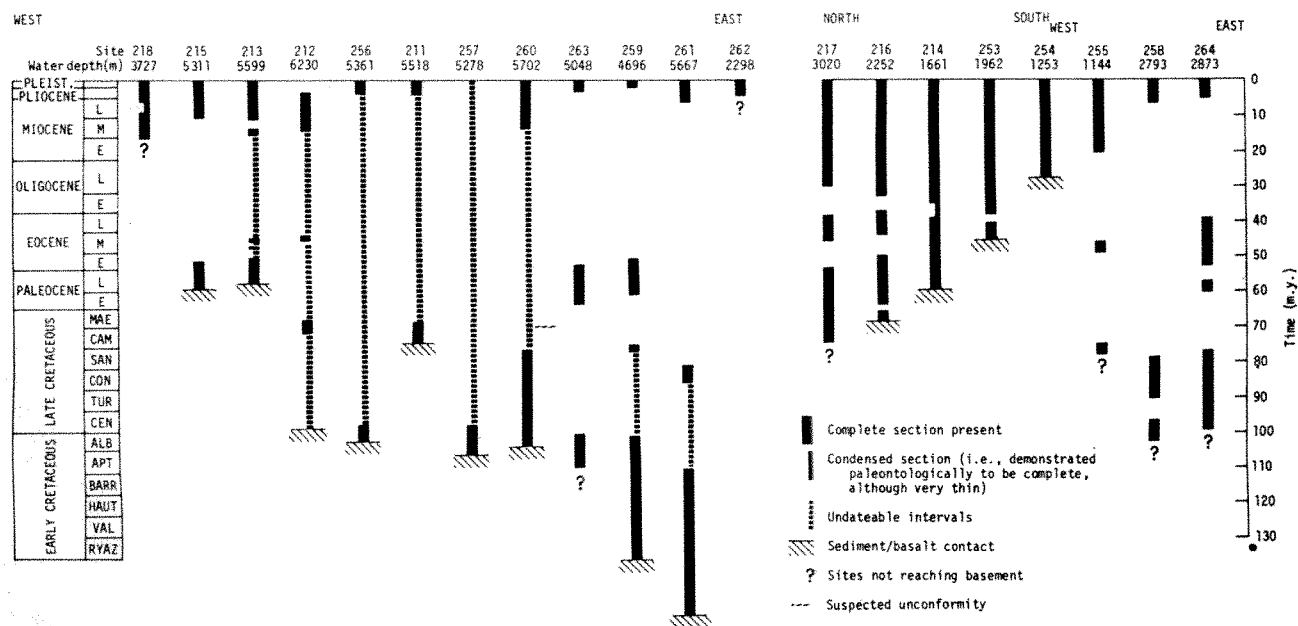


Fig. 3 Stratigraphic section sampled at sites in the eastern Indian Ocean. Ridge and plateau sites (right) are separated from the basin sites (left). The basement age for Site 212 is derived from geophysical evidence¹⁷.

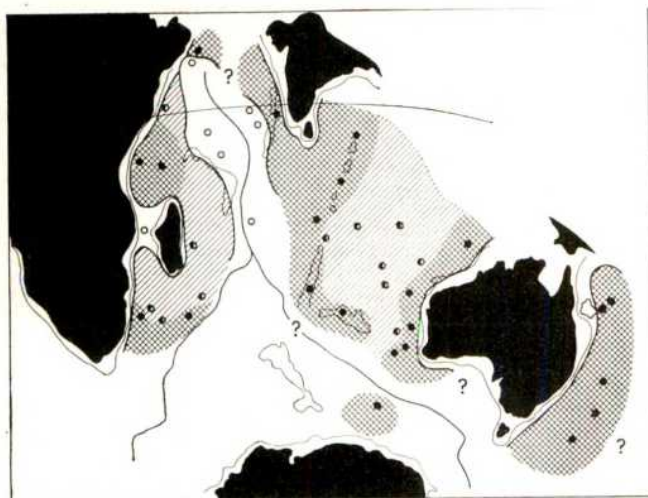


Fig. 4 DSDP sites plotted on an early Oligocene reconstruction of the Indian Ocean²⁶. Open circles represent sites where the sedimentary section is complete; solid circles where there is a proven unconformity, and half circles where there is an undated interval or an inferred unconformity. The shading indicates the extent of the Oligocene unconformity; crosshatching showing the extent of the proven unconformity.

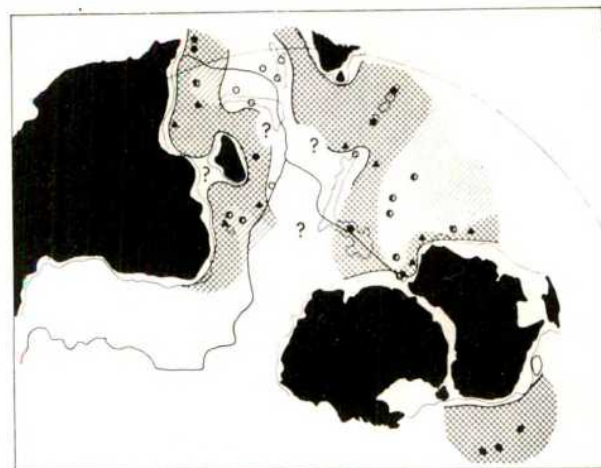


Fig. 5 DSDP sites plotted on a middle Eocene reconstruction of the Indian Ocean²⁶. ○, sites where the sedimentary section is complete; ●, sites where there is a proven early Tertiary unconformity; half circles where there is an undated interval or an inferred unconformity; ▲, sites where an early Tertiary unconformity cannot be separated from the overlying Oligocene unconformity. The shading indicates the probable extent of the early Tertiary unconformity; crosshatching showing the extent of the proven unconformity.

Significance of the hiatuses

Attempts to explain the cause or causes of these hiatuses must consider numerous variables, including tectonism and continental drift, world climate, oceanic productivity, and oceanic circulation and oceanic chemistry, all of which are inter-related in a complex way. In perhaps the simplest cases (the early Tertiary unconformity on Broken Ridge (Site 255) and those near Owen Ridge (Sites 223 and 224)) the unconformities are clearly the result of tectonic uplift and subsequent erosion^{2,17}. On the other hand Kent¹⁸ has drawn attention to the fact that hiatuses in the sediments of the south-west Indian Ocean, while seemingly corresponding to tectonic events on land, are more likely to have been the results of variations in oceanic circulation and sediment supply rather than being directly attributable to tectonic events. The same may be said of the gaps found in the sedimentary record on the Naturaliste Plateau; for the sites in the deep basins remote from tectonic activity the links between hiatuses and such activity must be even more tenuous, if not non-existent. Even if we exclude locations for which there is a clear case for a tectonic origin for the unconformities, we are still left with the task of explaining the occurrence of oceanwide gaps in the sedimentary record. Recognising the limited data available and the complexity of the problem, we will limit our speculations to the Oligocene and early Tertiary hiatuses.

The following observations might be considered facts regarding these hiatuses which require explanation:

- (1) Both hiatuses are expressed over a wide depth range.
- (2) Well developed unconformities appear in the western Indian Ocean and south-west Pacific whereas undated intervals, probably containing unconformities, are more common in the eastern Indian Ocean.
- (3) The early Tertiary and Oligocene hiatuses have similar regional extent in the Indian Ocean and south-west Pacific.
- (4) The Oligocene hiatus is not found in the central Pacific as is the early Tertiary hiatus.
- (5) The Oligocene unconformity in the Indian Ocean and south-west Pacific occurs in concert with high calcareous sedimentation rates in the South Atlantic and central Pacific.

In our discussion we use the following model for hiatus formation in the deep sea. True unconformities are due to erosion or non-deposition caused by vigorous surface and/or deep ocean currents. The fact that the hiatuses in question cover such a wide depth range indicates that both deep and surface circulation have been involved. Dissolution facies are

formed below the CCD where calcareous material is dissolved. The CCD is the level where dissolution of carbonate by cold, under-saturated bottom waters balances carbonate supply from surface waters. The CCD may be moved shallower by a decrease in regional productivity, removal of oceanic carbonate by precipitation on shelf areas, or an increase in the amount and rate of supply of cold bottom waters, all of which result in an increase in the under-saturation of bottom waters¹⁹.

Bottom water in the Southern Hemisphere forms in the Antarctic shelf regions, notably in the Ross and Weddell Seas²⁰. Weddell Sea water in part flows east into the south-western regions of the Indian Ocean where it behaves as a western boundary undercurrent. Bottom water also drifts north through fracture zones in the south-east Indian Ridge, where it may be joined by Ross Sea water flowing west between Australia and Antarctica, to pass through the gap between Naturaliste Plateau and Broken Ridge into the Wharton Basin²⁰. This pattern of circulation seems to mirror the regional development of the Oligocene hiatus and possibly the early Tertiary one as well, and would explain why the hiatuses are more definitely expressed in the western ocean regions. Tectonic and climatic events in the Antarctic region are therefore important.

An important event in the late Cretaceous was the rifting of New Zealand from Antarctica and the opening of the Ross Sea²¹. Shortly after this, in the early Eocene or before, climatic deterioration and glacial conditions occurred in Antarctica²². This combination of events may have led to the formation of substantial amounts of 'aggressive', erosive Antarctic Bottom Water, Ross Sea water entering the central and south-west Pacific to erode and dissolve sediments and Weddell Sea water spreading eastwards between Africa and Antarctica into the western basins and through the gap between Broken Ridge and Naturaliste Plateau into the Wharton Basin. Surface circulation was intensified as a result of climatic cooling and perhaps increased storminess. This could explain the occurrence of well-defined unconformities at many of the shallow sites contemporaneous with undated intervals and unconformities in the deep basins. If surface productivity and faunal diversity were also decreasing during the early Tertiary, as is widely believed, these would serve to enhance shoaling of the CCD and result in the extensive formation of dissolution facies.

A similar story can be constructed for the Oligocene hiatus since climate deterioration and late Oligocene glaciation are

probable for Antarctica at that time²². It is curious that the Oligocene hiatus is not found in the central Pacific. Post-Oligocene erosion is found in the Samoan gap, the bottom water conduit into the central Pacific²³. Dissolution of great quantities of carbonate in the Indian Ocean and south-west Pacific in the Oligocene is possibly related to the contemporaneous high carbonate sedimentation elsewhere in the Pacific and Atlantic as the ocean responded to the need to maintain a chemical balance. Also, it is possible that at this time Ross Sea water was confined to the south-west Pacific by Melanesia and thus had relatively little influence in the regions farther north.

Between the late early Eocene and the middle of the Oligocene climatic conditions in Antarctica were probably a little warmer. This was sufficient to reduce the supply of Antarctic Bottom Water, encourage increased surface productivity, and diminish the intensity of current activity to the point where sedimentation could resume. The Oligocene hiatus was brought to a close by the gradual establishment of the present pattern of circum-Antarctic flow sometime near the end of the Oligocene²⁴. This again reduced the inhibiting influence of Antarctic Bottom Water on sedimentation in the ocean basins farther north, since a substantial proportion of the Antarctic Bottom Water was diverted into circumpolar flow and proportionately less found its way into the basins.

This attempt to attribute regional patterns of unconformities to climatic events in Antarctica is incomplete in several respects. First, the present day pattern of Antarctic Bottom Water circulation and its effects on deep ocean sedimentation are not entirely clear, much less these effects 60 Myr ago. Palaeobathymetry models would be helpful, as would further hydrographic stations and piston cores in the Indian Ocean. Second, present understanding of what effect Antarctic glaciation had on ocean surface water movements is equally vague. These currents have probably contributed to forming the hiatuses at shallow sites. We recommend experimental and analytical study of these currents taking into account continental dispersion and palaeowinds²⁵. Finally, more precise evaluations of the stratigraphy at each site will result in a more refined picture of palaeocirculation patterns.

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Structure and assembly of filamentous bacterial viruses

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The protein coat of filamentous bacterial viruses is constructed from segments of a helix that extend in an axial direction, and overlap each other like shingles or fish scales to form a hollow cylindrical shell. This hollow shell contains the viral DNA.

THE protein coat of filamentous bacterial viruses^{1,2} is rich in α helix. This has enabled us to develop a model for the virus structure. We have used low resolution X-ray diffraction data to define the general arrangement of α helices, followed by molecular model building to define atomic positions. Our model

has implications for the morphogenesis of these viruses, and also for the structure of linear assemblies of proteins in general.

Filamentous bacterial viruses consist of linear assemblies of coat protein subunits encapsulating single-stranded, circular DNA. The virions measure about 60 Å in diameter by 10,000 to 20,000 Å long, depending on the strain, and contain no more than 12% by weight of DNA. The major coat protein in all strains comprises about 99% of the viral protein, has a molecular weight of about 5,000, and is largely α helical. Their relative simplicity and the relatively high quality of their X-ray diffraction patterns makes these viruses uniquely valuable models for the study of the molecular structure of fibrous proteins and

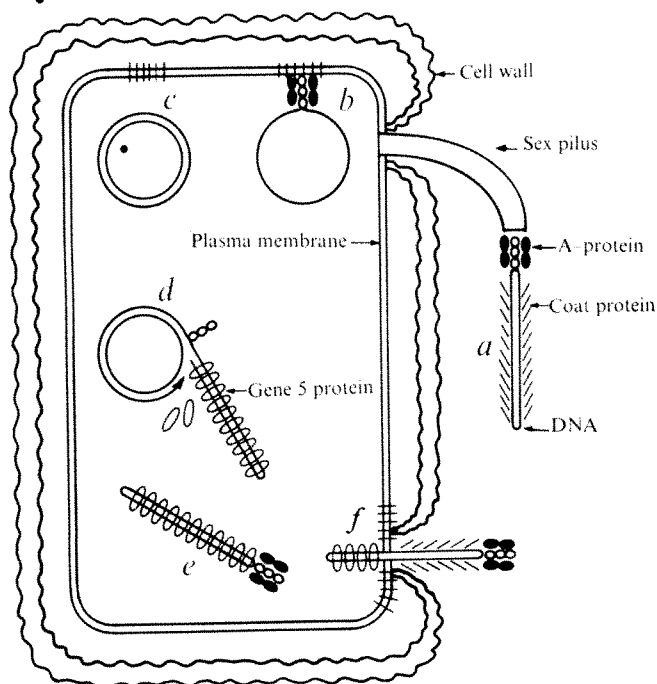


Fig. 1 Schematic illustration of some features of the filamentous bacterial virus life cycle. Successive stages are drawn counter-clockwise around the bacterium, although it is unlikely that all stages would be found at once in any given bacterium. The entire illustration is consistent with current data, but some features are less solidly supported by experiment than others. *a*, The virus attaches to the Gram-negative bacterium by means of a minor coat protein, the A protein (the bacterial attachment site is the sex-pilus for some strains² but not others^{28,29}). *b*, The viral DNA and A protein³⁰ enter the bacterium, leaving the major coat protein at the plasma membrane²⁷. *c*, The viral DNA is converted to a duplex form, that replicates to give several hundred progeny duplexes. Viral DNA replication may require the presence of viral coat protein in the membrane³¹, perhaps to create a new site for DNA replication³². *d*, The progeny duplex spins off a single-stranded tail that is coated with the protein product of viral gene 5. *e*, The DNA is closed to give a circular DNA molecule in a linear DNA-gene 5 protein complex³³. *f*, The viral DNA passes out through the bacterial envelope. The gene 5 protein is displaced from the DNA by coat protein that had previously been deposited at the plasma membrane²⁵. The completed virion is released from the bacterium without lysing or otherwise killing the bacterium³⁴. Inhibition of viral assembly in contrast does kill the host in a process involving the gene 5 protein³⁵. More complete citations of the earlier literature are given in ref. 2.

nucleoproteins. In addition, their unusual life cycle (Fig. 1) makes them interesting systems for the study of dynamic molecular interactions such as the displacement of one protein by another from a nucleoprotein complex, and the transport of macromolecules across membranes.

Structures of these viruses

Concentrated gels of filamentous bacterial virus can be oriented in fibres for X-ray diffraction studies³. Two classes of diffraction pattern, differing in detail, were found during a survey of various strains carried out in this laboratory^{4,5}. The class I pattern is given by the fd, f1, M13, If1, and IKe strains⁴; the class II pattern is given by the Pf1 and Xf strains⁵. The class II pattern is simpler to interpret, and has therefore been analysed in more detail⁶. The position of diffracted intensity on the pattern indicates⁶ that the protein subunits in the virus are arranged on a helix of pitch ~ 15 Å, with 4.4 subunits in one pitch length of the helix (Fig. 2). The relative magnitudes of diffracted intensity indicate that the diffracting material at each surface lattice point is elongated into a rod that makes an angle of about 20° with the helix axis, and also tilts from large to small radius in the virus. The orientation determined for the rods of electron density is such that rods originating on one turn of the helix interdigitate between rods originating on the next turn.

Spectroscopic measurements^{7,8} show that the α -helix content

of the coat protein approaches 100%. We have therefore assumed that the rods of electron density found from qualitative analysis of the X-ray pattern can be considered as single rods of α -helical protein measuring 10 Å by 70 Å, the dimensions of an α helix with molecular weight 5,000, and have used model-building techniques for further analysis of the structure. The α helix was represented in initial models by a row of points distributed on a curve 70 Å long. The Fourier transform⁶ of this repeat unit in the virus helix was calculated for various orientations and curvatures of the row, and compared with the class II diffraction pattern. The results of many trial calculations confirmed the conclusions about orientation of rods of electron density that were derived from direct analysis of the diffraction pattern. For the next stage in our model building, the row of points was replaced by a 70 Å polyalanine α helix. We generated the co-ordinates of atoms in this α helix by extending Crick's equation for the co-ordinates of a coiled coil⁹ to allow for a change in radius of the α helix in the major helix frame. Scattering factors of atoms were modified as described by Wilkins and coworkers¹⁰ to take into account the background electron density due to water and low density protein sidechains¹¹. The contribution of the DNA to the calculated diffraction was ignored at this stage.

Both intrachain and interchain stereochemistry were included as further constraints on the model. The intrachain stereochemistry of the α -helix rod was refined by fitting a stereochemically perfect polypeptide chain to the rough guide coordinates generated for the curved α helix, using the computer program developed by Diamond¹². The Ramachandran angles were $110^\circ < \phi < 133^\circ$ and $123^\circ < \psi < 149^\circ$ over the length of the refined α helix, and internal hydrogen bond lengths of the refined structure were also within the permissible range for α helix¹³. The interchain stereochemistry (the distance between

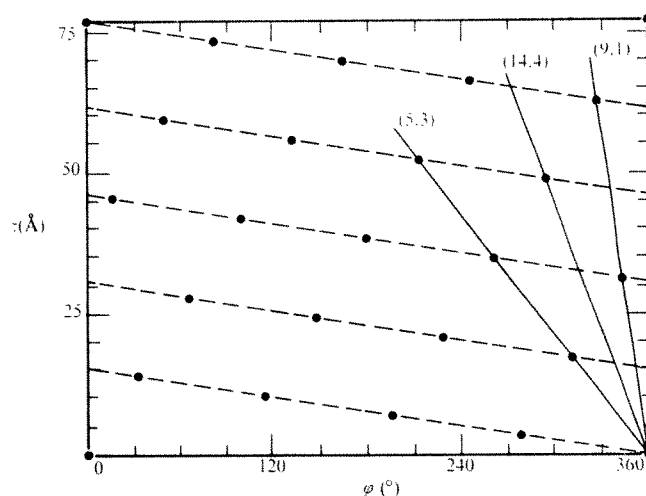


Fig. 2 Surface lattice of the class II helix. The lattice shows the 22 equivalent points in a 77 Å section of the virus helix, projected on to a cylindrical surface coaxial with the virus, which is then opened out flat and viewed from the outside of the helix. The dashed line shows the ~ 15 Å pitch of the virus helix. There are 22 units in 5 turns or 4.4 units per turn⁶. These helix parameters define the order of the Bessel functions predicted on each layer line of the helix diffraction pattern. In particular, for layer lines $l = 1, 2$ and 3 , the lowest order Bessel functions are those with order $|n| = 9, 4$ and 5 , respectively. The sign associated with $|n| = 5$ and 9 is opposite to that associated with $|n| = 4$. The sense of the helices with (n, l) indices $(5, 3)$ and $(9, 1)$ must be the same as that of the ~ 15 Å virus helix (they are shown as left-handed in this figure), but the absolute sense is not defined by the X-ray data. The fact that intensity is stronger on $l = 1$ and 3 than on $l = 2$ indicates that rods of electron density have the same sense as the ~ 15 Å helix. These rods of electron density roughly follow the $(14, 4)$ helix. The maxima of intensity on both $l = 1$ and $l = 3$ are observed at 0.1 Å⁻¹ along the layer lines, even though the predicted Bessel function orders are quite different for these two layer lines. This indicates that the rods of electron density tilt from large to small radius as well as slewing around the virus⁵.

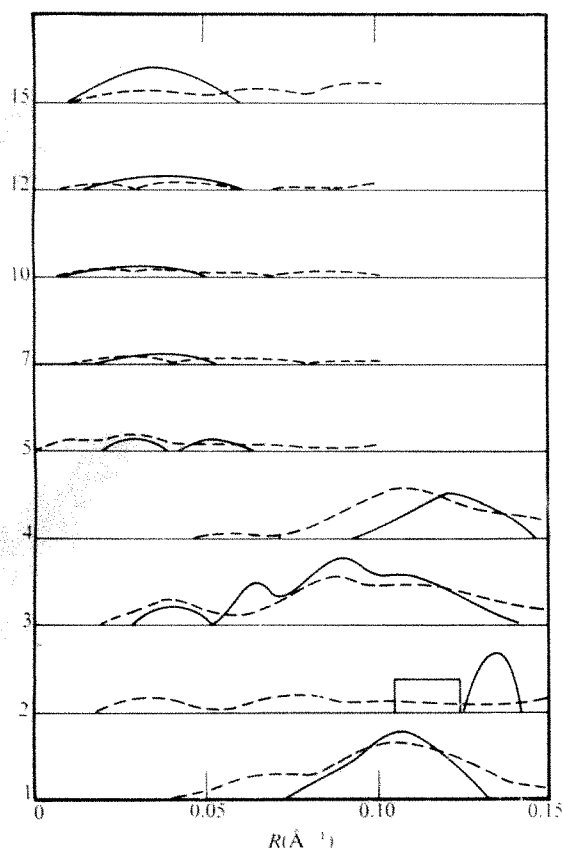


Fig. 3 Comparison of the calculated Fourier transform of the class II protein model with the observed diffraction amplitudes. Continuous diffraction on non-equatorial layer lines was measured^{13,15} on a diffraction pattern of Pf1 taken at 98% relative humidity by Dr R. L. Wiseman⁵ (film No. 2036). The cylindrically averaged Fourier transform was calculated for a repeat unit consisting of a 46-residue stretch of polyalanine α helix the axis of which roughly follows a segment of a conchospiral with $\alpha = -21^\circ$ and $\gamma = 11.5^\circ$ between 14 Å and 26 Å radius. This repeat unit was placed in a left-handed helix having 22 units in 5 turns in an axial repeat $c = 77$ Å. Over most of the region of close contact between neighbouring α helices, distances between the α -helix axes were 10 ± 1 Å and crossing angles between the axes were $9-12^\circ$. Observed amplitudes are solid lines; calculated amplitudes are dashed lines. The rectangular box on $l = 2$ represents integrated intensity for which the shape could not be determined. Analysis of the equator is discussed in ref. 15.

neighbouring α helices and the angle at which the axes of neighbouring helices cross each other) was such that sidechains on one α helix could fit into the space between sidechains on its neighbours in knobs-into-holes packing⁹. Only α helices whose axes follow a left-handed helical path can form satisfactory knobs-into-holes packing, indicating that the sense of the ~ 15 Å virus helix is also left-handed (Fig. 2).

The resolution of the X-ray data is not sufficient to define the positions of specific sidechains, but reasonable assumptions about the orientation of the protein molecule can be made on general chemical grounds. The acidic N-terminal end of the Pf1 protein was placed at the outer radius of the virus, and the basic C-terminal end at the inner radius, as first suggested for fd⁷. The α helix was then rotated about its own axis so that arginine-44 and lysine-45 were directed inwards, towards the virus core; the acidic residues were directed outwards at the virus surface; and most of the hydrophobic residues were involved in contacts with neighbours¹⁴.

There are narrow limits on the range of models that are consistent with all these stereochemical constraints and yet give calculated transforms similar to the observed. The observed and calculated transforms for the current best model are compared in Fig. 3. The model is illustrated in Figs 4 and 5.

Fourier analysis of the equatorial intensity (which gives information about the electron density projected down the long

axis of the virion) shows that the DNA occupies a central core surrounded by a shell of protein, with virtually no interpenetration of DNA and protein¹⁵. X-ray diffraction and chemical evidence, however, are insufficient for a detailed model of the DNA to be built at this time.

Symmetry and perturbation

An overlapping or shingled arrangement of axially elongated protein subunits as found for filamentous bacterial viruses is a stable and durable design for fibrous proteins. Larger bonding surfaces are available for interaction of subunits than would be available in a comparable structure made of globular subunits. In the minimum energy configuration¹⁶ for such an assembly, there are likely to be regular contacts along the length of neighbouring subunits, resulting in similar bonds at different radii. These bonds will be related by symmetry elements that operate not just in the surface lattice of the helix, but in three dimensions.

For the conformation derived experimentally for class II coat protein subunits, the α -helix axis roughly follows a segment of a conchospiral, a space curve with many interesting symmetry properties^{17,18}. The conchospiral is given by the equations

$$\begin{aligned} r &= a \exp(\phi \cot \alpha \sin \gamma) \\ z &= c \exp(\phi \cot \alpha \sin \gamma) \\ \tan \gamma &= a/c, \end{aligned}$$

where r, ϕ, z are cylindrical polar coordinates, α is the constant angle that the tangent to the curve makes with the z axis, and γ is the half-angle of the cone on which the conchospiral lies. This curve is the most symmetrical space curve aside from the circular helix. The curvature and torsion of the curve change continuously in a regular way as one progresses along the curve, giving regular internal symmetry. The segments of conchospiral in the filamentous virus helix are located so that each segment is roughly centred along its length between its nearest neighbours. There are two main kinds of intermolecular contacts: from any protein molecule to the proteins originating five and nine units, respectively, down the ~ 15 Å virus helix (Fig. 5). In the class II structure these contacts are sufficiently similar so that each protein in the helix has essentially equivalent environments in both directions, although a faint meridional reflection on the ~ 15 Å layer line indicates a slight perturbation repeating periodically about once per turn of the ~ 15 Å virus helix. The corresponding meridional reflection on class I patterns, and by implication the associated perturbation, is much stronger⁴. Contacts between the class I α helices that correspond to the 0 to -5 class II contacts in Fig. 5 are regular, but contacts corresponding to 0 to -9 are irregular, with a perturbation that repeats about every five units. This perturbed configuration presumably optimises local contacts and is therefore more stable than any unperturbed configuration that could be formed by the class I subunits.

Three principles for the design of linear assemblies of proteins are suggested by the detailed studies of filamentous viruses. (1) Linear assemblies will often be composed of axially elongated overlapping subunits. (2) Interdigitation of elongated subunits in neighbouring turns of the helical array will be facilitated if there are (roughly) an odd number of units in two turns of the helix. (3) Contacts between interdigitating neighbours will often be improved by a perturbation away from the ideal helical positions, repeating once every turn of the helix, and giving rise to a set of meridional reflections at orders of a periodicity that corresponds to the pitch of the helix rather than to the subunit spacing. Overlapping arrangements of elongated subunits have been proposed for myosin filaments in muscle¹⁹⁻²¹. Sharp meridional reflections have been observed on diffraction patterns of many fibrous proteins¹³, and in some cases have been interpreted in terms of perturbation. Some linear assemblies,

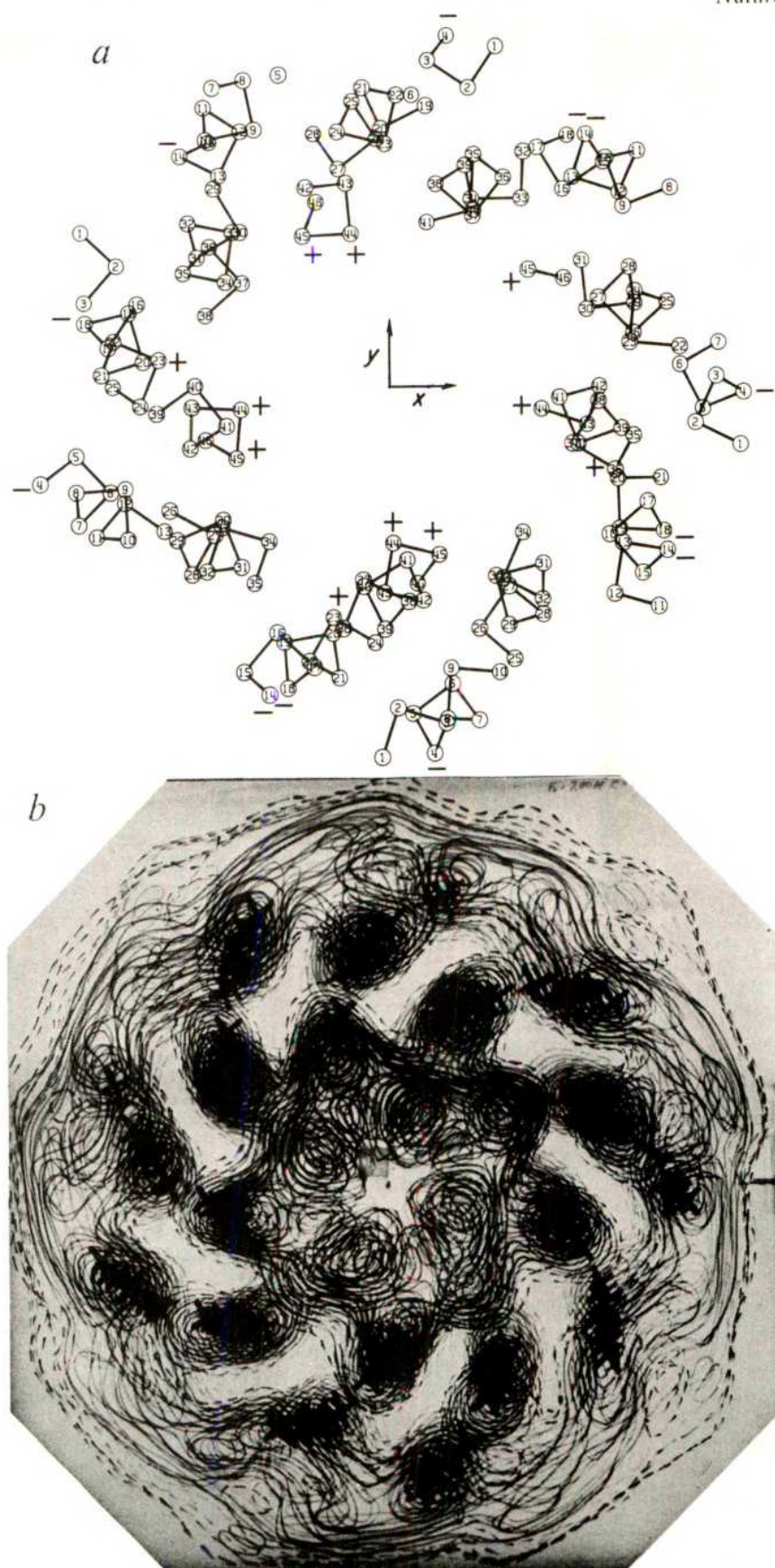


Fig. 4 Axial section of the class II model. A 14 Å slab through one virion, equivalent to four helix asymmetric units, is shown for convenience in visualising the packing of neighbours. *a*, Ball-and-stick representation of the α -carbon atoms, projected on to a plane normal to the axis of the virion. Numbers indicate residues. Several segments of different α helices overlap, but can be distinguished by discontinuities in the sequence of numbers. The basic residues (20, 44 and 45) and acidic residues (4, 14 and 18) are indicated by + and -, respectively. The box measures 30 Å on a side. *b*, Electron density in a corresponding slab, calculated⁶ using³⁶ the observed amplitudes and the phases of the protein model shown in Fig. 3. For the equator, phases were taken from Fig. 7*b* of ref. 15, and amplitudes from continuous transform of a non-crystalline Pf1 fibre at 100% relative humidity, measured and corrected for residual crystalline reflections as described in ref. 15. Crosses indicate the positions of axes of α helices in the model. Zero contours are dashed and may enclose negative density. Although DNA was not included in the phasing, it is expected that phases calculated on the basis of the protein model alone will be approximately correct, and may reveal structure not included in the model, such as possible phosphate peaks in the central DNA region.

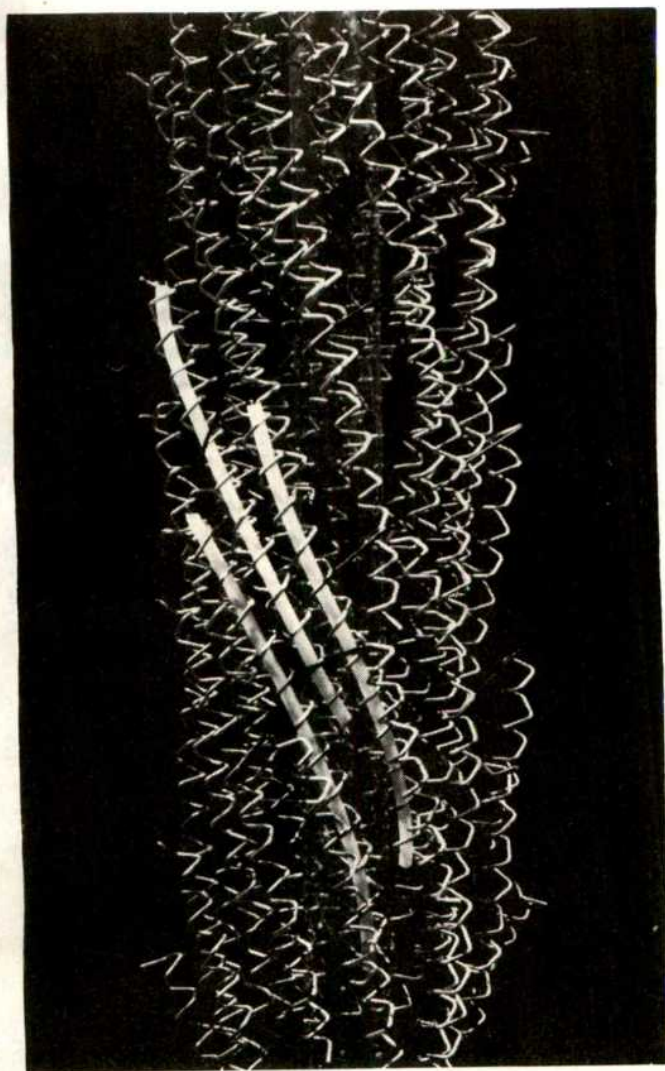


Fig. 5 Model of a 154 Å slab of the protein coat of the virus. The long axis of the virion is vertical. The protein subunits are represented by bent rod following the path from one α carbon to the next. Three neighbouring α helices are picked out by lengths of tubing: they are shown in the same view as Fig. 3 of ref. 14. The right-most α helix originates five units down the ~ 15 Å helix with respect to the central α helix, and the left-most α helix originates nine units down. Note the gentle curve of the α helices, and the interdigitation of neighbouring helices. DNA would occupy the central cylindrical cavity in the protein shell. The outer diameter of the lucite support rod is 6 Å in the model.

such as microtubules or bacterial flagella that have been assumed to be composed of globular subunits²²⁻²⁴ should perhaps be re-examined in the light of these principles.

Assembly

With the molecular structure of the virion in hand, we can discuss specific hypotheses about viral assembly. We assume the model of assembly illustrated in Fig. 1, and restrict ourselves to the steady-stage of assembly, after the forward end of the virion has passed across the membrane. The viral coat protein is a single rod of α helix with a hydrophobic central portion and hydrophilic ends. The protein could be excreted directly into the lipid bilayer²⁵ during synthesis²⁶, with its N-terminal end outwards and its long axis normal to the plane of the bilayer². The DNA, stripped of the gene 5 protein would, form a condensation centre for the coat protein previously stored in the bilayer. As the protein assembled on to the DNA, its hydrophobic areas would become masked, so that the protein would become less soluble in lipid than in water and therefore

would leave the lipid for the water, taking associated DNA with it. The process would be akin to crystal growth where the growing point of the crystal is fixed in space so that the crystal must move away from the growing point. The sense of the process (the virion moves out of rather than into the bacterium) would depend on the details of the configurational change in the coat protein upon assembly. A similar process in reverse could drive the dissolution of protein off the virion into the lipid bilayer²⁷ and advance the DNA into the bacterium during infection. This model—that assembly of hydrophobic proteins within a lipid bilayer causes changes in the properties of the completed assembly so that it becomes more soluble than the monomer in an aqueous phase and thus moves out of the bilayer—could apply in general to transport of oligomeric or polymeric proteins through membranes.

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letters to nature

Measurement of circular polarisation in the Crab Nebula at 1,415 MHz

RECENTLY Rees and Gunn¹ suggested that the 30-Hz wave power probably emerging from the Crab Nebula Pulsar NP0532 does not propagate further than a small region around the pulsar with a radius of about 10% of the nebular radius. Beyond this, in the main body of the nebula, the magnetic field is built up in toroidal form to equipartition strength so that the continuum emission from the bulk of the nebula is entirely synchrotron radiation. The axis of this toroid is aligned with the rotational axis of the pulsar which is assumed to be lying in the plane of the sky along the major axis of the nebula (NW/SE). A test of this proposal is the measurement of a characteristic sign change in the distribution of Faraday rotation or circular polarisation over the nebula. If the nebula is divided into quadrants along its major and minor axes, then adjacent quadrants should have the opposite sign and opposite quadrants the same sign. Since any measured Faraday rotation does not necessarily arise within the body of the nebula, the critical test becomes a detection of the characteristic sign distribution in the small amount of circular polarisation ($\sim 0.1\%$) expected at radio frequencies from ordinary synchrotron radiation². The theoretical frequency dependence of $\nu^{-1/2}$ for the fraction of circular polarisation would then explain the null result of Landstreet and Angel³ (see also ref. 4) at optical frequencies. Here I report a measurement of the distribution of circular polarisation (Stokes parameter V) in the Crab Nebula at 1,415 MHz obtained during a series of high accuracy full polarisation measurements with the Westerbork Synthesis Radio Telescope in November 1971.

A brief description of the normal method of polarisation measurement with the Synthesis Telescope can be found in ref. 5. In our case, this was modified by having, in each of the 20 simultaneous interferometers, the crossed dipoles in both telescopes parallel rather than at the usual 45 degrees to each other. The corrections for instrumental polarisation of the order of 1% of the total intensity were determined by observing 3C48 for 2×12 h and assuming that it was totally unpolarised at 1,415 MHz. It has been shown by Conway *et al.*⁶ ($V = +0.015 \pm 0.010\%$ at 1,415 MHz) and by Berge and Seielstad⁷ ($V = -0.024 \pm 0.024\%$ at 1,420 MHz) that this assumption is accurate for the V component with which I am concerned here. The final accuracy of the measurement of the distribution of circular polarisation in the Crab Nebula was then approximately 0.02% of the total intensity.

The great radio flux of the nebula overloaded all interferometer baselines shorter than 300 M and these had to be eliminated from the observation. Thus, the circular polarisation map is mainly sensitive to the smaller scale features in the source and not to the broader extended structure.

The resulting map of the distribution of circular polarisation in the Crab Nebula is presented in Fig. 1. The vertical and horizontal scales refer to the position of the pulsar (the cross). The r.m.s. noise error in the map is approximately one contour. Because of the missing short interferometer spacings, the area of the synthesised beam is not well defined and a conversion of the brightness contours to units of temperature will not be attempted. Even though V can assume both positive and

negative values, the negative feature to the west of the pulsar cannot, in this case, be distinguished from the effect of the missing short spacings. Also, since its magnitude is only about twice the r.m.s. noise error in the map, I do not consider it to be a real negative component of circular polarisation. Similarly, although feature A on the map cannot be generated by the effect of missing short spacings, it is only twice the r.m.s. noise error and thus of marginal significance.

For comparison with the distribution of total intensity, Fig. 2 shows the total intensity map from Duin and van der Laan⁸ obtained with the Westerbork Synthesis Telescope in a special detuned observing configuration which avoided the saturation problems discussed above. The frequency of observation and the resolution are the same as in Fig. 1 and a direct comparison of the maps can be made. From this it is possible to compute the circular polarisation for the four lettered areas as 0.04% (A), 0.05% (B), 0.06% (C) and 0.05% (D). These values can only be rough estimates due to the possibility of an undetected larger scale background in the source. They are, however, consistent with the amount of circular polarisation

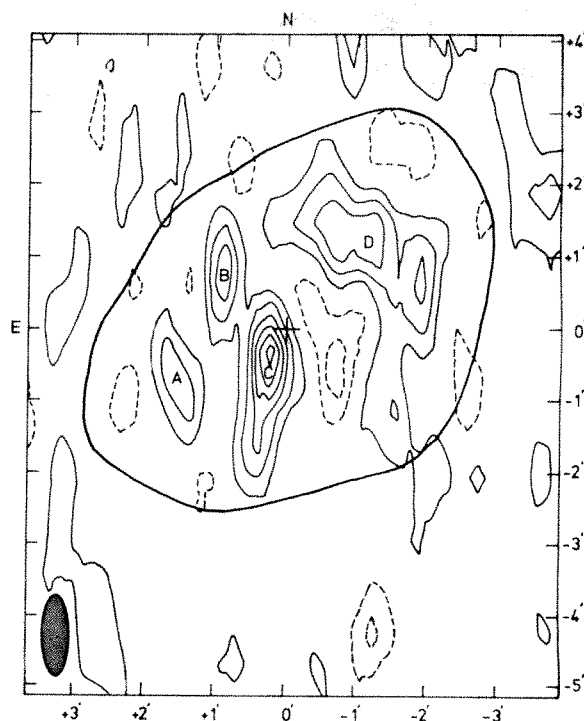


Fig. 1 Contours of the circular polarisation (Stokes parameter V) for the Crab Nebula at 1,415 MHz. The contour interval is 2.5 mJy per synthesised beam area. Positive contours are solid and represent right-hand circular polarisation (IRE definition). Negative contours are dashed and no zero contour is plotted. The heavy outside contour (taken from Duin and van der Laan⁸) is where the total intensity has 10% of its peak value. The cross marks the position of NP0532 (α (1950.0) = 05 h 31 min 31.46 s; δ (1950.0) = $21^\circ 58' 54''.8$). The half-power synthesised beam width is shown in the lower left corner.

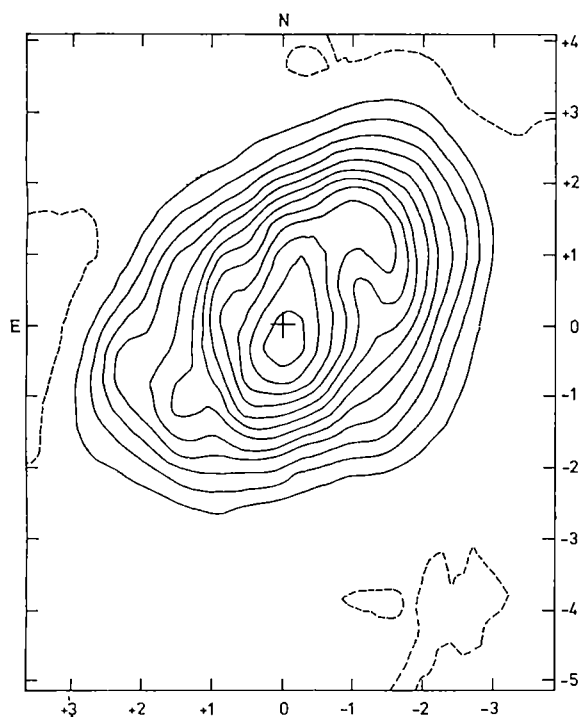


Fig 2 Contours of the total intensity (Stokes parameter I) at 1,415 MHz from Duin and van der Laan⁸. The contour interval is 2 Jy per synthesised beam area and the peak brightness is 25 Jy per synthesised beam area. The zero contour is plotted dashed. The cross marks the position of NP0532. The resolution and horizontal and vertical scales are the same as in Fig 1.

expected from normal synchrotron radiation in a static magnetic field of 10^{-3} to 10^{-4} gauss.

The major feature of Fig 1 is that whenever circular polarisation is detected, its sign is positive, indicating an average line-of-sight component of the magnetic field directed towards us. There is no indication of the sign reversal between adjacent quadrants expected from a toroidal structure.

The simplest magnetic field structure consistent with the present measurements is that of a relatively uniform field of 10^{-3} to 10^{-4} gauss directed with its line-of-sight component towards us. This form and strength is then quite similar to that suggested by Weiler and Seielstad⁹ from synthesis measurements of the linear polarisation and Faraday rotation at radio wavelengths. An effective mechanism for maintaining and amplifying such a field during the lifetime of the nebula is, however, still needed.

An independent confirmation of the circular polarisation in the Crab Nebula would be extremely useful. Thus, it should be noted that the presence of only positive values of V in the synthesis map implies that the integrated radiation should have a detectable amount of circular polarisation at radio frequencies. This could be of the order of 0.05–0.1% at 1,415 MHz and theoretically more at lower frequencies. There is only one previous measurement of the fraction of circular polarisation in the integrated radiation of the Crab Nebula at radio wavelengths which exceeds 0.1% in accuracy. Seielstad and Weiler⁷ found $+0.13 \pm 0.09\%$ circular polarisation at 1,418 MHz from measurements with the Owens Valley interferometer. Although this is insufficiently larger than the errors to be trusted, it is interesting that it agrees in sign with the present measurements.

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Nucleosynthesis and matter–antimatter cosmologies

If light elements are of cosmological origin, then a given cosmological model should be able to reproduce the observed abundances¹. Symmetric matter–antimatter cosmologies^{2–4} do not stand up to this test too well, and encounter problems whose basis is the annihilation process itself.

Steigman⁵ has emphasised that there is an important loss of neutrons at temperatures of the order of 1 MeV. (The matter–antimatter separation is assumed⁴ to have taken place at temperatures of the order of 350 MeV.)

Protons are essentially 'frozen' within a matter region by electrostatic interactions, and do not diffuse towards the matter–antimatter interface, this, however, is not true of neutrons, which are affected only by the interactions of their magnetic moment with that of the electrons (for which the cross section is very low) and by elastic collisions with protons. When the temperature has dropped below 1 MeV, weak interactions can no longer maintain an equilibrium between the protons and neutrons, and the neutron abundance in a region within a diffusion length of the interface ($\sim \sqrt{Dt}$), where D is the neutron diffusion coefficient and t the time, will drop as a consequence of the annihilation which occurs there.

It follows that the neutron–proton ratio, on which nucleosynthesis depends, is essentially a function of the ratio of the neutron diffusion length to the mean size of a matter region. Now, according to Omnès⁶, the size of a matter region turns out to be of the order of a neutron diffusion length: the neutrons must therefore all escape and be annihilated. Under these conditions primordial nucleosynthesis cannot take place.

Nevertheless, this estimate for the typical size of a matter region is not necessarily the only possible one, since there is some considerable uncertainty about the physical processes which occur at temperatures above 1 MeV, one might therefore try to fix empirically the 'size of the emulsion' (the somewhat misleading term often used to denote the size of a matter or antimatter region) by requiring that the symmetric model give the observed abundances.

To do this, we have to modify the usual evolution equation for the neutrons⁶, since the annihilation follows diffusion. If we suppose that all neutrons which reach the interface are annihilated, the evolution equation can be written as

$$dn/dt = \lambda p - \hat{\lambda} n - (Dt/4\pi)^{1/2} n/Lt \quad (1)$$

using the notations of ref. 6, D being the neutron diffusion coefficient and L the emulsion size (L is a function of temperature). The proton evolution equation is written in the usual way, since the diffusion is negligible

$$dp/dt = \hat{\lambda} n - \lambda p \quad (2)$$

If we suppose that L varies with temperature as $L = L_1 T^{-1}_{\text{MeV}}$ cm, we can integrate equations (1) and (2) to obtain a lower limit for the emulsion size at $T=1$ MeV that is, $L_1 \gtrsim 3 \times 10^6$ cm. The calculations have been done using various other equations for L (simple power laws, a sequence of different power laws joined piecewise), and it turns out that the smallest emulsion size is obtained by taking account only of expansion. This is the smallest emulsion size for which annihilation does not affect the relative neutron abundance: therefore, at the end of nucleosynthesis ($T \sim 0.1$ MeV), the light elements will have their usual¹ abundances.

At this epoch, the Universe is a matter-antimatter emulsion consisting for the most part of protons and helium nuclei together with the corresponding antielements. Although nucleosynthesis has finished, the annihilation processes themselves continue: an antiproton reaching the interface can annihilate against a proton or one of the nucleons within a helium nucleus, producing in the latter case a deuteron together with a number of neutrons. The deuterons produced in this kind of reaction will have a large kinetic energy, will leave the annihilation zone and, being charged, will be slowed down rapidly in the matter region. We shall call this 'direct deuterium'. It can be shown that all the neutrons will also be thermalised by elastic collisions with protons and helium nuclei, note, however, that this process is somewhat slower. As soon as they are sufficiently slow, they are captured by the protons to create more deuterium—this will be referred to as 'indirect deuterium'. The capture cross section rises very rapidly at low energies.

The annihilation rate per unit surface depends only on the nucleon flux at the interface, and so is independent of the emulsion size. Therefore, the annihilation rate per unit volume is inversely proportional to L : the smaller L , the larger the number of annihilations $\bar{p}-\alpha$ and the larger the amount of deuterium produced. Now, we must not at this stage produce too much deuterium (subsequent stellar destruction cannot lower the amount by an order of magnitude)—deuterium formation after the end of nucleosynthesis introduces therefore a new constraint on the emulsion size.

To calculate the amount of deuterium produced by this process, we have to know the branching ratios of the reactions $\bar{p}-\alpha \rightarrow d + \text{anything}$ and $\bar{p}-\alpha \rightarrow n + \text{anything}$. Since very little experimental and theoretical information is available, the following very rough model has been used. We suppose that the low energy ($E_{\text{kin}} \leq 1$ MeV) antiproton annihilates against the first nucleon which it meets in the ${}^4\text{He}$ nucleus, and produces an average of 5–6 pions. Experiment⁷⁻¹⁰ suggests that, on average, only one of these pions interacts with the remaining nucleons, which are still sufficiently close together to be treated as a ${}^3\text{He}$ or ${}^3\text{H}$ nucleus. The reaction $\pi-{}^3\text{He}$ (ref. 11) has been studied experimentally for pions at rest, and so the number of deuterons produced per interaction (~ 0.25) is probably overestimated and the number of neutrons (~ 1.75) underestimated. There could be ~ 7 times as much indirect deuterium produced as direct if each neutron were captured. Detailed calculations¹², which take into account the path lengths of the various different particles, show that to a first approximation we may in fact neglect the direct deuterium.

An appreciable quantity of deuterium will be produced only if the capture process $p+n \rightarrow d+\gamma$ be more important than the photodissociation $d+\gamma \rightarrow p+n$, that is if the temperature be below 0.07 MeV. We shall denote the time at which this temperature is reached by t_i . This production stops when the temperature is so low that the neutron decay has become more important than capture: this occurs at the time t_f . The deuterium abundance is therefore calculated for the interval $t_i - t_f$.

$$d/p = \int_{t_i}^{t_f} (\nu L^2 B) \times \frac{1}{2} \frac{75}{dt/pL^3} dt$$

where d and p are the number densities of deuterium and protons respectively, ν is the $\bar{p}-p$ annihilation rate (it can easily be shown that $B \sim \alpha < \sigma v >_{\bar{p}p} / < \sigma v >_{pp}$, where α is the number density of helium, the other quantities having their usual meaning¹³), $L = L_1 T^{-1}_{\text{MeV}}$ is the emulsion size. Note that we have introduced a factor $\frac{1}{2}$ because, on average, one half of the products of a $\bar{p}-\alpha$ annihilation pass into a matter region, and one half into an antimatter region.

In order that this process does not produce more deuterium than is observed^{1,14} $d/p \lesssim 10^{-5}$. The emulsion size at a temperature of 1 MeV is therefore given by $L_1 \gtrsim 1.5 \times 10^8 (\Omega h'^2)^{1/3}$ cm, where Ω is the density parameter and $h' = H_0/50$ (H_0 is the Hubble constant in $\text{km s}^{-1} \text{Mpc}^{-1}$).

During the radiative era, the nucleon number density rises by a factor of $\sim 10^3$ as a consequence of annihilation, any subsequent annihilation will therefore affect the element abundances very little.

So the amount of helium and deuterium produced as a consequence of nucleosynthesis can be made to agree with observed abundances only if the emulsion size at 1 MeV exceeds the neutron diffusion length by a factor of about 10^3 (at this epoch, $\sqrt{Dt} \sim 10^4$ cm).

Nevertheless, it would at this stage be unwise to discuss the future of symmetric cosmologies. Our present state of knowledge does not allow us to calculate the emulsion size in any definitive way.

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Of fundamental electrodynamics and astrophysics

MAXWELL's equations are well established for phenomena on laboratory and terrestrial scales, but there are other electrodynamic theories, as yet indistinguishable on such scales from Maxwell's theory, that have significantly different consequences for phenomena on an astronomical scale. For example, extensive magnetic fields are possible in the Universe¹ because of the general absence of magnetic monopoles. Parker¹ considered the abundance of magnetic monopoles that would neutralise or dissipate the magnetic fields of the Earth, Sun and the Galaxy, and deduced for the number density of free magnetic monopoles upper limits that are more stringent than those deduced from laboratory and terrestrial data.

Another example of electromagnetic equations that might be relevant to the description of astrophysical phenomena are the Proca equations, which may be interpreted in terms of a non-zero photon rest mass. A consequence of the Proca equations is that static electric and magnetic fields in free space decay exponentially with distance from the source. For phenomena on scales comparable with or exceeding the characteristic length for decay, the description provided by the Proca equations departs from that provided by the Maxwell equations. In static space-time the Proca equations, like the Maxwell equations, predict that electromagnetic waves propagate with fixed frequency, contrary to what has been claimed^{2,3}, the Proca equations do not form a basis for the 'tired-light' interpretation of the cosmological redshift.

Laboratory and terrestrial experiments have determined⁴ that the photon rest mass, m , is less than about 3×10^{-48} g, astrophysical considerations have led to an improvement by about four and a half orders of magnitude⁵. Here, we obtain an upper limit on m from consideration of the Proca equations and the mass of the Galaxy.

The Proca equations show that $(\mathbf{E}^2 + \mathbf{H}^2 + \mu^2 \phi^2 + \mu^2 \mathbf{A}^2)/8\pi$ can be interpreted as the energy density of the electromagnetic field⁴, \mathbf{E} and \mathbf{H} are the electric and magnetic fields, ϕ and \mathbf{A} are the scalar and vector potentials and $\mu^{-1} = h/2\pi mc$, where h is Planck's constant and c is the relativistic limiting speed. If ρ denotes an upper limit on the mean mass density of matter and energy in all forms that could exist in a region of space in which a magnetic field is observed, the $\mu^2 \mathbf{A}^2/8\pi c^2 \lesssim \rho$. The relationship $\mathbf{H} = \Delta \times \mathbf{A}$ shows that $|\mathbf{A}| \sim |\mathbf{H}| L$ where, for an approximately uniform magnetic field, L is of the order of the smallest dimension. Thus, the above inequality leads to

$$\mu^2 \lesssim 8\pi \rho c^2 / \mathbf{H}^2 L^2 \quad (1)$$

The Galactic magnetic field in the vicinity of the Sun has been observed⁶ to have a strength of about 2×10^{-6} gauss and is approximately uniform over a distance of at least 300 pc, so that in the Galactic disk L may be estimated as greater than about 10^{20} cm. The masses of galaxies may be estimated from their rates of differential rotation and from the orbital velocities of binary systems, it has been deduced⁷ that for many galaxies the mass exceeds the total mass of known stars by an order of magnitude. The mass of the Galaxy is often quoted⁸ at about $10^{11} M_\odot$ so that $10^{12} M_\odot$ is a conservative upper limit on the mass of the Galactic disk. Thus, using the dimensions given by Allen⁸, $\rho \lesssim 10^{-21}$ g cm⁻³ for the Galactic disk. Assuming that the interstellar magnetic field in the vicinity of the Sun is typical of magnetic fields of the Galactic disk, equation (1), with $|\mathbf{H}| \sim 2 \times 10^{-6}$ gauss, $L \gtrsim 10^{20}$ cm and $\rho \lesssim 10^{-21}$ g cm⁻³, leads to $\mu \lesssim 3 \times 10^{-14}$ cm⁻¹, corresponding to $m \lesssim 10^{-51}$ g. This limit is not far from the best available upper limit⁵ of 10^{-55} g.

In interstellar space the energy densities resulting from radiation from stars, turbulent gas motions, universal background radiation and cosmic rays are all of a similar order of magnitude to the Maxwellian energy density $\mathbf{H}^2/8\pi$, associated with the

magnetic field⁸, if $m \sim 10^{-52}$ g, then the energy density $\mu^2 \mathbf{A}^2/8\pi$ exceeds these energy densities by 11 or more orders of magnitude. It may well be that the upper limit of 10^{-52} g could be improved by consideration of the possible sources of the energy density, $\mu^2 \mathbf{A}^2/8\pi$, associated with large scale magnetic fields and of instabilities that may result from this energy density. Conversely, it is worth bearing in mind that large energy densities associated with large scale magnetic fields and a non-zero photon rest mass could be significant in some astrophysical processes.

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Oblique rotators in binary systems

RADIO pulsar PSR1913+16, which has a period of about 59 ms, has been identified as a member of a binary system¹. The X-ray 'oscillars' HerX-1 and CenX-3, which are also members of binary systems, are not radio pulsars (I use the word 'oscillars' to distinguish these objects from pulsars, which maintain their periodicity much more accurately). These facts, together with the 34-d periodicity^{2,3} of HerX-1 and the lack of this periodicity in CenX-3, can be explained by using the rotating neutron star hypothesis for the compact object in all of these systems. As is common in pulsar theories, I assume that rotating neutron stars are oblique rotators, that they have magnetic fields and that the magnetic axes are not along the axes of rotation.

An oblique rotator, with a magnetic field, B , a period of rotation, P , and radius, R , gives out low frequency radiation with energy⁴

$$E \simeq 10^{-28} B^2 R^6 / P^4 \text{ erg s}^{-1}$$

Using $B \sim 10^{12}$ gauss, $R \sim 10^6$ cm and a period, P , of 59 ms, PSR1913+16 gives out about 5×10^{37} erg s⁻¹ of low frequency radiation. This is quite close to the Eddington limit and can equal it with only a slight variation of B or R . Radiation of this intensity opens the equipotential lines near the first Lagrangian point⁵, and accretion of matter from the binary companion is thus prevented. The radio emission, which is attributed to a polar phenomenon⁶ can, therefore, occur without interference from accreting matter. The lack of accretion prevents PSR1913+16 from becoming an X-ray oscillator like HerX-1 or CenX-3. On the other hand, PSR1913+16 could be an X-ray pulsar like NP0532, but with considerably smaller flux.

HerX-1 has an X-ray periodicity of 1.24 s and if this is attributable to the rotation period of the neutron star, the low frequency radiation emitted is about 5×10^{31} ergs s⁻¹. CenX-3 has a period of 4.8 s and gives out about 2×10^{29} erg s⁻¹.

These values for energy emission are considerably smaller than the Eddington limit and so matter could accrete on to the neutron star. If the radio emission process is to continue, that matter must absorb the radio radiation by the free-free process. This can be verified by considering the rate of accretion implied by the X-ray emission of HerX-1 and CenX-3. Thus, it can be seen why HerX-1 and CenX-3 are not radio pulsars.

The low frequency radiation emitted by HerX-1, though not large enough to prevent mass accretion, still regulates it in an interesting manner. Suppose that the density of matter at a distance r from a neutron star of mass, m , ($\sim 1M_{\odot}$) is ρ . Then, equating the radiation pressure and the gravitational force

$$GM\rho/r^2 = 5 \times 10^{31}/4\pi r^2 c$$

where G is the gravitational constant and c is the velocity of light. This gives $\rho \simeq 10^{-6} \text{ g cm}^{-3}$, and thus in the case of HerX-1 unless the density is higher than about $10^{-6} \text{ g cm}^{-3}$, accretion cannot occur. The matter accreting from the binary companion piles up at the light cylinder and when the density exceeds the calculated value accretion starts and continues until the density again reaches the critical value. Thus, it could be taking 22 d to accrete enough matter to reach the required density, and 12 d for all the released matter to spiral into the neutron stars, during which time X-ray emission occurs. That would also allow the emission of X rays during the 22-d quiescent period, as has been observed⁷. For when the companion star ejects matter as, for example, in a flare, the density in the reservoir may exceed the required value in less time than the average accretion time and so X-ray emission could occur.

In the case of CenX-3, the emission of low frequency radiation is much smaller than in HerX-1, and only low densities of matter are needed to overcome the radiation pressure. Therefore, accretion can occur all the time.

Another interesting effect on the oblique rotator in a binary system is the oscillation of the magnetosphere of the neutron star. This occurs because of the gravitational force of the companion star on the matter in the magnetosphere, somewhat similar to the tidal effect on Earth. This makes the lines of force, which are assumed to be frozen in, vibrate as the neutron star rotates. By calculation I found that in the case of HerX-1 the fluctuation of the field intensity is 100% at around a few times 10^9 cm . The effect of this fluctuation is to accelerate particles. But the energy involved is only about 10^{32} – $10^{33} \text{ erg s}^{-1}$. A detailed account will be presented elsewhere.

Note added in proof Margon *et al.*⁸ observed PSR1913+16 for an hour using the Copernicus satellite, for X-ray emission in the region 1–3 Å. They did not find any X-ray flux and give an upper limit to $10^{-10} \text{ erg cm}^{-2} \text{ s}^{-1}$. The non-observation of X-ray flux from PSR1913+16 is consistent with the model suggested above, since the low frequency radiation prevents accretion.

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Polarisation of atmospheric bremsstrahlung

THE terrestrial atmosphere is bombarded continuously by energetic charged particles of extraterrestrial origin. When a charged particle penetrates deep into the atmosphere, either its direction changes or its speed varies with respect to the local phase velocity of electromagnetic waves. These changes, caused by the dense atmosphere at altitudes of about 90–100 km, generate bremsstrahlung X rays. The flux of atmospheric bremsstrahlung X rays has been measured using balloons^{1–3}, rockets and satellites^{4,5}. Measurement of the atmospheric X-ray flux has become routine in the study of electron flux precipitation into the lower ionosphere^{6,7}. The degree of polarisation of emitted radiation changes rather drastically with the changing energy of precipitating electrons and with the height of precipitation. But no effort has yet been made to measure the polarisation of atmospheric bremsstrahlung. We here show the potential importance of bremsstrahlung X-ray polarisation and its probable role in atmospheric diagnostics.

In the auroral zone electron penetration is almost vertically downwards. Electrons penetrating the dense atmosphere give rise to linearly polarised bremsstrahlung X-ray photons. The degree of linear polarisation is a function of the kinetic energy of scattering electrons, the energy of the emitted photons, the angle of photon emission with respect to the direction of the incident electron, and the atomic number density of atmospheric gas. The classical and quantum mechanical theories of linear polarisation of bremsstrahlung X rays have been fully developed. The success of a diagnostic technique is based primarily on the compatibility between experimental measurements and theoretical computations. But the theoretical computation of the degree of linear polarisation using rigorous quantum mechanical theory, and the detailed nature of target structure have posed a serious problem. This has inhibited the use of the degree of polarisation as a diagnostic measure. Kirkpatrick and Wiedmann⁸ have developed an approximate computational procedure for obtaining the degree of polarisation as a function of the projectile and target parameters. Using that procedure we have computed the height profile of the degree of polarisation of bremsstrahlung X rays in the terrestrial atmosphere and have shown its variation with related parameters.

Bremsstrahlung X rays received at a point in the z - x plane (z being vertical), at an angle θ relative to the incident electron direction can be resolved into two linearly polarised intensity components, I_{\perp} and I_{\parallel} . The radiated X ray is assumed to be linearly polarised in the direction of the emitting equivalent

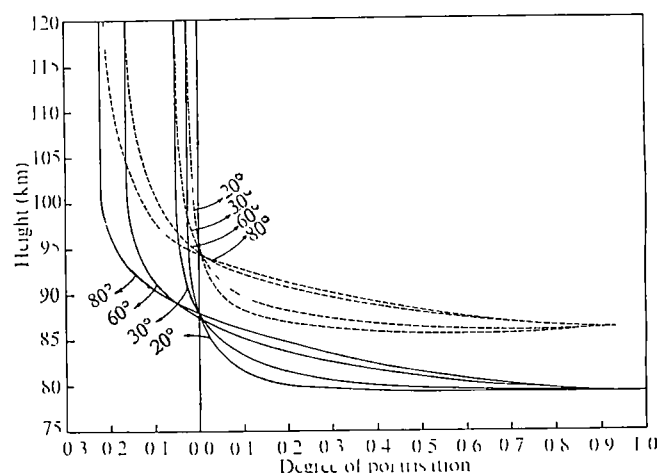


Fig. 1 Variation of the degree of polarisation with height of 20 and 40 keV electrons (dashed and solid lines, respectively) for different angles of photon emission.

dipole. Therefore, I_{\perp} will have only a y component whereas I_{\parallel} will have x and z components. Thus

$$\begin{aligned} I_{\perp} &= I_y, \\ I_{\parallel} &= I_x \sin^2\theta + I_z \sin^2(90^\circ - \theta) \end{aligned} \quad (1)$$

For an unpolarised electron beam there exists an axial symmetry so that

$$\begin{aligned} I_y &= I_z, \\ \text{and } I_{\parallel} &= I_x \sin^2\theta + I_y \cos^2\theta \end{aligned} \quad (2)$$

From equations (1) and (2) the degree of linear polarisation can be defined as

$$P(\theta) = \frac{(1 - I_y/I_x)}{[(I_y/I_x)(2\csc^2\theta - 1) + 1]} \quad (3)$$

By this definition the degree of polarisation, $P(\theta)$, ranges from +1 (complete polarisation perpendicular to the electron-photon plane) to -1 (complete polarisation parallel to that plane).

In view of the difficulties involved in the computation of I_x and I_y using rigorous quantum mechanical theory we have resorted to an approximate method following Kirkpatrick and Wiedmann⁸

$$I_x = 10^{-50} (Z^2/V) [0.252 + a((v/v_0) - 0.135) + b((v/v_0) - 0.135)^2] \quad (4)$$

and

$$I_y = I_z = 10^{-50} (Z^2/V) [-j + k/(v/v_0 + h)] \quad (5)$$

where v_0 and v are the photon frequencies corresponding to the initial and final energies of the scattering electrons, V is the velocity of electrons in e.s.u. and Z is the atomic number of the scatterers. The constants a , b , h and k are empirical constants defined by Kirkpatrick and Wiedmann⁸

The height profile of V/Z^2 and v/v_0 have been obtained from detailed computation of the energy degradation of charged particles into the ionosphere^{9,10}. I_x and I_y are functions of the height profiles, V/Z^2 and v/v_0 which give rise to the height profile of $P(\theta)$ (Fig. 1). It is obvious from Fig. 1 that measurement of the degree of polarisation, $P(\theta)$, at a particular height is very sensitive to changes in the energy of incident electrons and to the direction of the emitted photons. The height of the transition of the plane of polarisation from + to - changes with the changing incident energy of the electrons. The height of the transition is, however, independent of the angle of bremsstrahlung photon emission. This variation with height suggests that simultaneous measurements at different heights could allow the prediction of degradation of electron energy and changes in electron flux. The lack of a high efficiency, high resolution X-ray spectrometer has been the main reason for the paucity of experimental measurements of the polarisation of bremsstrahlung radiation produced by low energy electrons. The advent of the Ge(Li) detector has solved this problem and it seems quite feasible that the measurement of polarisation could be used as a diagnostic probe for studying precipitating electron energy and flux.

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Landau damping and cyclotron resonance in uniform magnetoplasma

The formation of a ledge in the electron distribution functions during Landau damping produces steep gradients at the boundaries of the disturbed region of the distribution function. This makes the pitch angle distribution sufficiently anisotropic to amplify a gyroresonant whistler mode perturbation at the appropriate frequencies.

The system under consideration consists of a homogeneous, collisionless hot plasma in a uniform magnetic field B_0 , a spectrum of electron plasma waves with their propagation vectors k antiparallel to B_0 , and a wide band whistler mode perturbation with k parallel to B_0 . For simplicity, the particle distribution f is taken to be initially independent of pitch angle $\alpha = \tan^{-1}(v_{\perp}/v_{\parallel})$ where v is the particle velocity and the subscripts \parallel and \perp refer to the vector components along and perpendicular to B_0 .

The electron plasma waves would be Landau damped with a growth rate¹ γ_L varying as $\partial f / \partial v_{\parallel}$ at $v_{\parallel} = v_{res}$, where $v_{res} = \omega_p/k$ is the Landau resonant velocity, ω_p being the electron plasma frequency. Simultaneously, the electron distribution would evolve quasilinearly in accordance with the equation¹

$$\partial f / \partial t = (\partial / \partial v_{\parallel}) [\{ (4\pi e^2 / m^2 v_{\parallel}) (E_k^2 / 8\pi) \} (\partial f / \partial v_{\parallel})] \quad (1)$$

where e and m are the electron charge and mass respectively and $E_k^2 / 8\pi$ is the energy density of the wave.

The growth rate² γ_c for the whistler mode perturbation, which results from cyclotron resonance with electrons is proportional to $(A(V_R) - B)$ where

$$A(V_R) \propto \left[\int_0^{\infty} v_{\perp} dv_{\perp} \{ v_{\parallel} (\partial f / \partial v_{\perp}) - v_{\perp} (\partial f / \partial v_{\parallel}) \} v_{\perp} / v_{\parallel} \right]_{v_{\parallel} = V_R} \quad (2)$$

and $B = \omega / (\Omega - \omega)$ with $V_R = (\omega - \Omega) / k$ and $\Omega = eB_0 / mc$

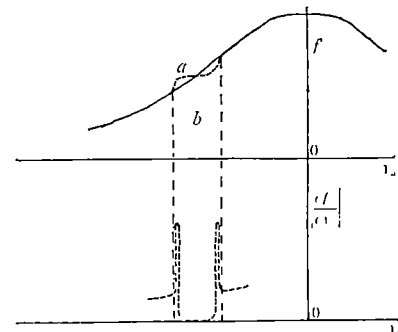


Fig. 1 Upper part f against v_{\parallel} , k is negative. The ledge (a) forms with negative values of v_{\parallel} (b). Disturbed region. Lower part $\partial f / \partial v_{\parallel}$, at different points of the ledge, against v_{\parallel} . The steep rise in f at the boundaries of b gives the peaks in $|\partial f / \partial v_{\parallel}|$.

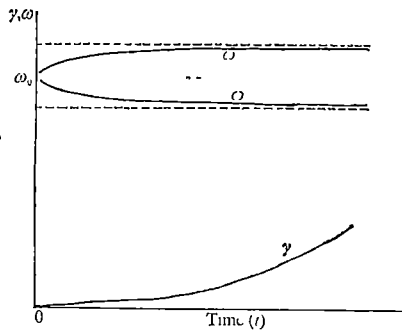


Fig 2 Behaviour of growing waves with time. Upper part frequency growing fastest at time t , against t . The curve has two branches, one above and one below the central frequency. Lower part maximum growth rate at time t , against t .

The cyclotron growth of the perturbation, in turn, affects the pitch angle distribution of the particles, which then evolves in accordance with the equation² (for $\omega \ll \Omega$)

$$\partial f(v, \alpha, t) / \partial t = (\pi e^2 / m^2 \sin^2 \alpha) (\partial / \partial \alpha) \{ (\sin \alpha B_z^2 / |v \cos \alpha|) (\partial f / \partial \alpha) \} \quad (3)$$

where $B_z^2 / 8\pi$ is the magnetic energy density of the whistler mode wave. Since $\partial f / \partial \alpha$ and $\partial f / \partial v_{||}$ are not independent, the resultant effect of the electron plasma and whistler mode waves on the electron distribution in the range where V_R and v_{res} overlap can be obtained by solving equations (1) and (3) simultaneously. Exact analytical solution of these equations is, however, very difficult and therefore, it would be worthwhile to look qualitatively into the behaviour of these equations.

For simplicity, only the initial stage of the growth of the whistler mode perturbation is considered, when its amplitude is too small to affect the background distribution significantly. For $\omega \ll \Omega$, $\gamma_c \propto A(V_R)$ and V_R is always negative. For a monotonically decreasing f with increasing $|v_{||}|$ and v_{\perp} , equation (2) gives

$$A(V_R) \propto \left[\int_0^\infty v_{\perp}^2 \partial v_{\perp} \{ |v_{\perp}| / |v_{||}| \} \{ \partial f / \partial v_{||} - |\partial f / \partial v_{\perp}| \} \right]_{v_{||} = V_R} \quad (4)$$

For an initially isotropic distribution $A(V_R) = \gamma_c = 0$ at $t = 0$. The quasilinear diffusion process caused by electron plasma waves leads to the formation of a ledge in f over the range of the resonant velocities. During this process $|\partial f / \partial v_{||}|$ diminishes at the centre but develops peaks on either side of the ledge (Fig 1), which grow in height and drift in position towards the boundaries of the disturbed region (Fig 2). As $\partial f / \partial v_{\perp}$ does not change during Landau resonance, the changes

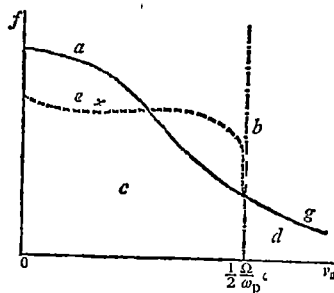


Fig 3 a, f against $v_{||}$ at $t = 0$, b, largest $v_{||}$ for Landau resonance, c and d, the resulting disturbed and undisturbed regions, respectively, e, the ledge formed over c after quasilinear relaxation, g unchanged f over d. The steep gradient, $\partial f / \partial v_{||}$, that develops near $v_{||} = c\Omega/2\omega_p$ would increase the pitch angle anisotropy and lead to whistler growth.

in $A(V_R)$ and γ_c (at corresponding frequencies) would follow those in $|\partial f / \partial v_{||}|$.

If ω is not much less than Ω , then γ_c will not become positive unless $A(V_R) > B$, which means that in the initial stage Landau damping would be accompanied with the cyclotron damping, and only after a certain time, when $A(V_R)$ exceeds B , would cyclotron growth occur.

This mechanism may play a significant role in the generation of natural and artificially stimulated very low frequency (vlf) emissions. If a wide spectrum of oblique whistler turbulence³ exists in the magnetosphere for a period of the order of the Landau resonant quasilinear relaxation time, a ledge is likely to form over the entire disturbed region in the velocity space. The whistler dispersion $c^2 k^2 / \omega^2 = \omega_p^2 / \{ \omega (\Omega \cos \theta - \omega) \}$ relation shows that the phase velocity has a maximum, $c\Omega/2\omega_p$, at $\omega = \Omega/2$. Therefore, a particle with $v_{||}$ greater than this would not be in Landau resonance with the waves and would, consequently, lie outside the disturbed region (Fig 3). A particle with $v_{||} = c\Omega/2\omega_p$ would gyroresonate with a whistler mode wave with a frequency of $\omega = \Omega/2$, propagating anti-parallel to B .

The steep gradient in the curve of f against $v_{||}$ which is consequently formed at $v_{||} = c\Omega/2\omega_p$ would, therefore, be unstable to whistler mode perturbations at $\omega = \Omega/2$ propagating in the negative z direction as equation (4) suggests and wave amplification may be expected at this frequency. This amplification would, however, be delayed as $A(V_R)$ would take some time to exceed B in order to give a positive growth rate. The nature of these results would not be altered even if the initial distribution were assumed to have an anisotropy in pitch angle.

Very low frequency emissions occur more often at half the equatorial gyrofrequency than at other frequencies³, it is likely that the mechanism described here is one that favours emissions at that frequency.

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Origin of the Naturaliste Plateau

LYING off the south-west coast of Australia (Fig 1) some 2,500 m below sea level is the broad, rectangular Naturaliste Plateau, which forms a relatively flat ledge half way between sea level and the abyssal plain. How the plateau came to be in this position, and whether its origin is continental or oceanic, are two questions relevant to the evolution of the south-east Indian Ocean.

Until recently the only data bearing on the origin of the plateau were seismic profiles recorded by RV Vema¹ and by RV Robert D. Conrad, which also obtained 2 m of Upper Cretaceous sedimentary rock² by gravity coring (RC8-56). Deeper core information was obtained in late 1972 by the Glomar Challenger at Deep Sea Drilling Project sites 258 and 264 (refs 3 and 4, and Fig 2). Preliminary study of DSDP data indicates that the plateau has been at its present depth since Early Cretaceous (Middle Albian) time, accumulating pelagic sediments, and 'has probably been uplifted since that time rather than having subsided'³.

DSDP 258 penetrated 525 m of marine sediment up to 105 Myr old, containing detrital units in the Early Cretaceous which may be volcanically derived and which support an oceanic origin. But a Miocene/Santonian unconformity

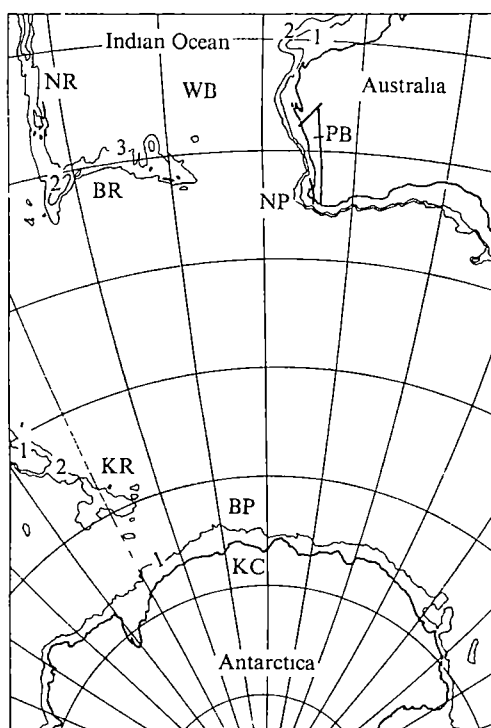


Fig. 1 Location of the Naturaliste Plateau (NP) in the south-east Indian Ocean BR, Broken Ridge, NR, Ninety-east Ridge, WB, Wharton Basin, KC, Knox Coast, BP, Bruce Plateau, KR, Kerguelen Ridge, PB, Perth Basin Bathymetric contours in km after McKenzie and Solater¹¹

which represents a break of 70 Myr was encountered only 100 m below the seafloor and may be related to the final separation of Australia and Antarctica in Middle Eocene time. In the Robert D Conrad core (RC8-56) an unconformity separates Pleistocene and Turonian beds.

DSDP 264 near the southern edge of the plateau penetrated 215 m of carbonate sediment and bottomed in an undated volcanoclastic conglomerate containing both acid and basic volcanic rocks. The Tertiary/Cretaceous unconformity at the other core sites is here represented by three unconformities spanning late Miocene/late Eocene, early Eocene/mid-Palaeocene, and mid-Palaeocene/Cenomanian times. The erosion causing these shallow unconformities seems to have been less severe at the margins of the Plateau, as evidenced by the presence of Eocene and Palaeocene sections at site 264.

The first systematic survey of the area was achieved when the Australian Bureau of Mineral Resources (BMR) completed a series of 13 profiles over the plateau in December 1972. This was part of BMR's survey of the Australian continental margin using continuous gravity, magnetic, seismic, and bathymetric recording at a line spacing of 30 nautical miles. A preliminary study of these data together with previous information suggests a continental origin for the plateau.

The western and eastern halves of the Naturaliste Plateau differ significantly in bathymetric, seismic, and magnetic characteristics. The western half is the shallower and is characterised by shallow seismic basement and intense magnetic anomalies. The basin-like eastern area has thick sediments and less intense magnetic anomalies. Along the southern and parts of the western margin, but not along the northern margin, scarp-like bathymetric features suggest faulting in the basement. Elsewhere the seismic results show that the plateau sediments are not faulted or folded. The thickest sedimentary sequence is in the north-west

corner and in the low area to the east, where at least 2 km of sediment is present.

The Tertiary/Cretaceous unconformity is too close to the sediment-water interface at drilling sites 258 and 264 for detection on BMR seismic records. But three deeper unconformities were revealed. All four (A, B, C, and D) are shown on the cross section along XX' shown in Fig 3. The Tertiary/Cretaceous unconformity (A), detected in all three plateau cores, also occurs at Broken Ridge and Wharton Basin Sites³, and is of regional extent. Unconformity B extends over the whole plateau and truncates unconformity C, restricting it to the eastern part. The sediment/acoustic basement unconformity (D) is most readily identifiable in the areas of thin sediments in the west.

The well stratified sequence above unconformity B constitutes about half the total volume of sediments evident on the plateau and shows several local unconformities. Its thickness ranges from 200 to 1,100 m, assuming a sediment seismic velocity of 2.0 km^{-1} and DSDP data indicate that it is largely of Cretaceous age with only about 100 m of

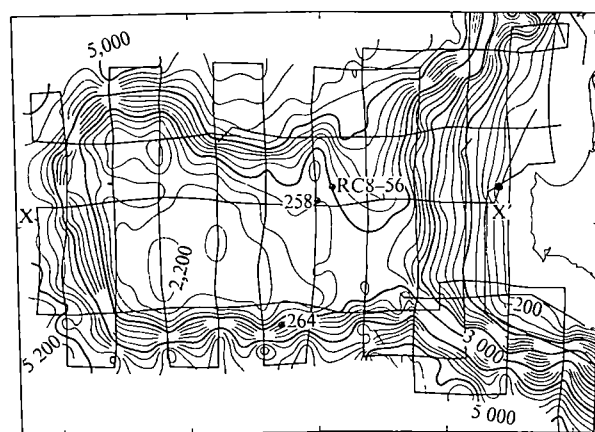


Fig. 2 Bathymetry of Naturaliste Plateau, BMR traverses and core locations RC8-56, core by RV Robert D Conrad, 258, DSDP core site 258, 264, DSDP core site 264. Bathymetric contour interval 200 m.

Miocene and younger beds. Most sedimentation on the plateau, therefore, preceded the separation of Antarctica and Australia in Eocene time.

DSDP 258 was near the pinch out between unconformities B and C, and bottomed just short of C in a thick sequence of Albian clays and glauconitic sands. Thus, the beds between B and C, which reach a thickness of 700 m in the deep eastern area, are evidently these Albian clays. On the seismic sections they are largely transparent, but stratification becomes increasingly evident northwards.

Unconformity C seems likely to be Neocomian because it is close to the Albian clays at the bottom of DSDP 258 and because prominent prior tectonic activity known in the area took place in the Perth basin within Neocomian time. Jones and Pearson⁵ believe this to be the time when India became detached from the west coast of Australia during the breakup of Gondwanaland. But recent DSDP data from site 259 in the Perth Abyssal Plain indicated that this fracturing continued throughout the Cretaceous⁶. The basalt at the base of DSDP 259 was dated as Neocomian.

Below unconformities B in the west and C in the east are well stratified sediments ranging in thickness from 0 to 1,000 m. In the west they are draped over the basement, in the east they extend to the limit of seismic penetration. They are only gently folded and faulted, and were deposited before the Cretaceous activity.

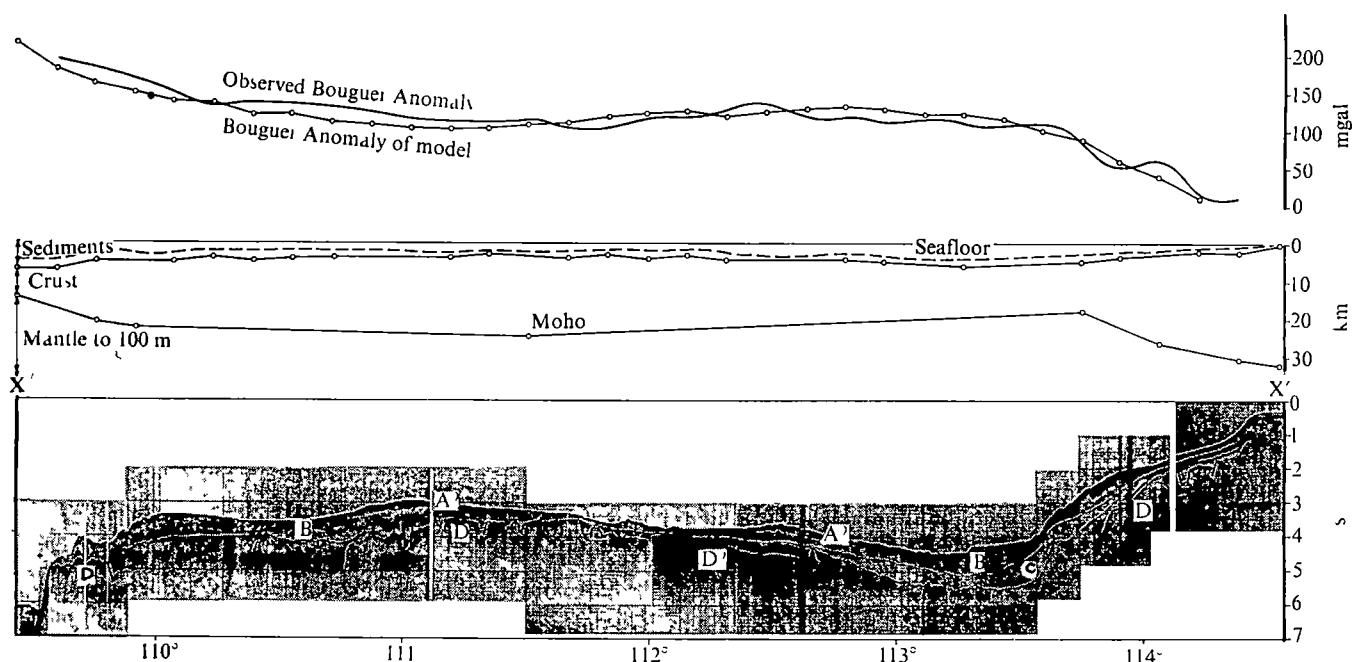


Fig. 3 Bouguer anomaly crustal model of the Naturaliste Plateau and seismic interpretation for section XX'. Densities used in gravity model: mantle 3.45 g cm^{-3} , crust, 2.85 , sediments, 2.20 . Standard crust for south-west Australia taken as 31 km thick after Mathur¹². A, B, C, D, are unconformities ↓ DSDP site 258

Acoustic basement is more visible on the seismic records from the western parts of the plateau, where sediments are generally thinner than in the east. Basement topography resembles a block-faulted and eroded plateau. Occasional reflection events below the basement unconformity suggest that the sequence is sedimentary, and this may be supported by the lack of strong reflectivity contrast with overlying sediments in some areas. Elsewhere there are features in the seismic sections showing intrusive form.

The magnetic anomaly pattern is more disturbed in the west, indicating a shallower magnetic basement than in the east. The high frequency and amplitude of magnetic features in the west suggest that the basement in this region is a complex of basic igneous and metamorphic rocks. This, together with the seismic evidence, points to a basement of metamorphosed sedimentary rock with basic igneous intrusions.

The Bouguer anomaly model showing the best fit with observed gravity is illustrated in Fig. 3. Crustal thickness is about 22 km , intermediate between continental and oceanic thicknesses, and is a further indication that the plateau was not originally an oceanic feature. A similar thickness was found⁷ under Broken Ridge from seismic refraction surveying in 1962 and it was suggested then that this feature is continental and may have been contiguous in the past with the Naturaliste Plateau and the Western Australian Shield.

BMR and DSDP data indicate that most of the plateau sedimentation preceded the separation of Australia and Antarctica and a large part was almost certainly pre-Cretaceous. The sediments were deposited on a basement of metamorphic rocks, and have remained tectonically undisturbed except along the southern and western margins. It is concluded that the plateau is of continental origin and existed as part of the continental mass of Gondwanaland in essentially the same position relative to Australia that it occupies today. Location of marginal scarp features suggests that there were landmasses along its southern and western margins, but not along its northern margin.

The reconstruction of Gondwanaland by Veevers *et al.*⁸ places India against the south-west coast of Australia and the Naturaliste Plateau some 500 km north of its present position. The reconstruction was based on evidence that the Perth Basin was within the interior of Gondwanaland up to the earliest Cretaceous. The BMR and DSDP data cast doubts on this reconstruction. The western basement block of the plateau could not have moved great distances horizontally relative to Australia since the deposition of the sediments without causing sedimentary deformations in the east that would be unmistakable in seismic records.

In the fit of Australia and Antarctica proposed by Sproll and Dietz⁹ the Naturaliste Plateau lies against a similar Antarctic marginal bathymetric feature known as the Bruce Plateau, offshore from Knox Coast. Rocks outcropping on Knox Coast are shown on the Geological Map of Antarctica¹⁰ to be Precambrian gneisses and schists of granulite facies. As suggested by the geophysical evidence such rocks are more likely than oceanic igneous rocks to form the basement of the Naturaliste Plateau. There is reason to believe, therefore, that the plateau once formed part of the Gondwanaland continental shelf (which explains the intermediate crustal thickness) abutting the Bruce Plateau. It broke away from Antarctica in the initial rifting which formed the southern Australian continental scarps and thereafter it remained attached to the Australian crustal plate.

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The contribution of palaeosoil to Egyptian lithostratigraphy

THOROUGH examination of the contact between the basement complex (mainly Precambrian) and the overlying Nubia Sandstone (Upper Cretaceous) in seven localities in the Eastern Desert of Egypt (Fig 1) has revealed the existence of a well defined lateritic palaeosol capping the former rocks. Previously, mention has only been made¹ of a local kaolinised surface of the basement rocks and the lateritic palaeosol has been neglected in the lithostratigraphy²⁻⁴. The nature, thickness (4-10 m remaining after erosion) and the lateral extent of the soil over a wide variety of rock types necessitates, however, the introduction of a separate unit, which we suggest is called the 'I'byan Soil'. This palaeosol may cover a large area of Egypt and we suggest that the same nomenclature is used at all localities where the soil has lateritic affinities.

At the type locality, about 1 km north of the 97-km post east of Idfu (longitude 33° 44'E and latitude 25° 2' 30"N), the 'I'byan Soil' is 10 m thick and caps the Barramiya Serpentinities (Fig 2). In other localities it caps either old volcanics (Fig 1, locality 2), arenites of the

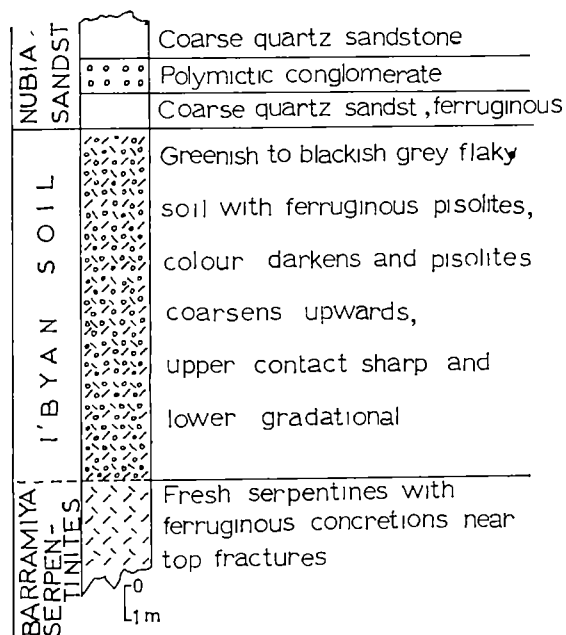


Fig 2 The succession at the type locality

Hammamat Group (Fig 1, locality 3), granodiorite (Fig 1, locality 4) or metasediments and metavolcanics (Fig 1, localities 5-7). The exact nature of the weathering of the basement rocks before the sandstone was deposited is now being studied through detailed geochemical and mineralogical analyses.

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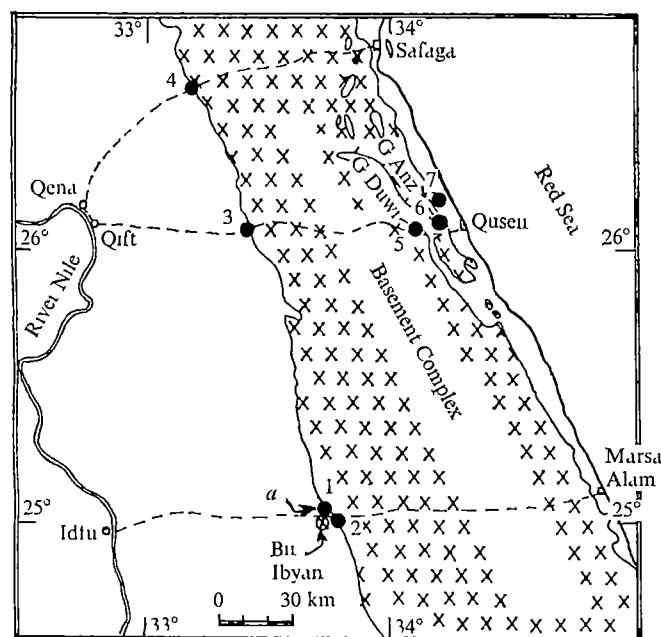


Fig 1 Locality map showing the visited localities of the 'I'byan Soil'. Roads are shown as dashed lines a, Type locality

Decreased global rainfall during the past Ice Age

We have made a synthesis of atmospheric conditions for 20,000 yr BP based on fairly standard palaeoclimatological data and procedures. From mean zonal conditions at 20° latitude intervals from 80°N to 80°S we have computed radiative heating rates and the energy balance of atmospheric columns. We find that net cooling of the atmosphere by radiative processes was smaller at 20,000 yr BP than it is now. The total heating of the air, which sums to zero in the annual average except during periods of climatic change, is the sum of heating by latent heat liberation, heating by conduction and convection from the boundary layer, and radiative cooling (see for example Fig 7 in ref 1). Because the change in radiative cooling is comparable to boundary layer heating itself, we postulate that the decrease in cooling is accompanied primarily by a decrease in heating from rainfall and therefore that global rainfall was smaller at 20,000 yr BP than it is now. Examination of the magnitudes of the three components suggests that the decrease in rainfall was about 10%.

Details of the palaeoclimatic reconstructions will be published elsewhere but for completeness we state here the chief assumptions made and give the radiative cooling rates found

Surface air temperature maps for January and July were synthesised from observation of oxygen isotopes in ice cores, observations of tree lines, snow lines, pollen distributions and foraminifera distributions. Variation of temperature in the vertical was assumed to have the same form as today. Specific humidity was taken as a function of temperature only, the relationship in the troposphere being based on present-day observations. Stratospheric moisture and ozone concentrations were taken as identical to present-day values. Carbon dioxide was assumed to have a uniform distribution of 320 parts per million by volume. Cloud was assumed to be absent everywhere. A set of zonally averaged albedo values was drawn up from a consideration of the nature of the surface at each point on a $10^\circ \times 10^\circ$ grid. Radiative heating rates, containing contributions from both the solar and terrestrial portions of the spectrum, were computed following the technique devised by Rodgers² and modified by Dopplack³. Meridional cross sections of these rates were drawn up and integrations performed to obtain the total cooling of the air by radiative processes. These integral values, for the present atmosphere with cloud and without cloud and for the 20,000 yr BP synthesis without cloud, but with and without the albedo change, are shown in Table 1.

Table 1 Integral over the entire atmosphere of the net radiative cooling rate

	Present day		20,000 yr BP	
	With cloud	Without cloud	No albedo change	New albedo
January				
Globe	10.4	11.2	10.6	10.4
Northern Hemisphere	5.9	6.1	5.6	5.6
Southern Hemisphere	4.5	5.1	5.0	4.8
July				
Globe	10.5	13.1	11.8	11.7
Northern Hemisphere	4.6	6.7	5.5	5.3
Southern Hemisphere	5.9	6.4	6.3	6.4

Units: 10^{20} calorie d^{-1}

The present-day values show greater cooling without cloud than with cloud. Inspection of the cross sections shows that when the cloud is removed some regions at middle latitudes alter from slight radiative heating to radiative cooling. It therefore seems reasonable to interpret this difference as due to the blanketing effect of the cloud. When we compare the results without cloud for 20,000 yr BP and the present, it is evident that there is a smaller net cooling at 20,000 yr BP, substantially all due to changes in the Northern Hemisphere. Most of this change is due to temperature and specific humidity changes, rather than to albedo changes. The latter influences the solar input radiation to the ground-atmosphere system but has no effect on the terrestrial radiation lost to space. Treating the air alone, there is less cooling in the 20,000 yr BP synthesis because temperature and moisture content were both lower.

The air itself is able to maintain an annual equilibrium temperature because the net radiative cooling is counteracted by latent heat liberation and boundary layer heating (see Fig. 7 of ref. 1). These values are respectively about 9.0 and 1.5×10^{20} calorie d^{-1} for the present day atmosphere. A decrease in radiative cooling of about 1.0×10^{20} calorie d^{-1} , for the 20,000 yr BP synthesis, must have been accompanied by a decrease in net heating by the same amount. We postulate that a large fraction of this decrease occurred in the latent heat liberation term and that the rainfall was therefore smaller, by roughly 10%. The values in Table 1 suggest that most of this change occurred in the Northern Hemisphere.

There have been many discussions in the past when it was argued that Ice Ages were 'pluvials,' which had the connotation of additional rainfall. The above comments suggest that rainfall was less then than now. Recently some palynological records from the tropics have also been interpreted to imply that 20,000 yr BP was actually a drier time than the present⁴.

Kraus⁵ has also argued for a decreased rainfall based upon a presumed lower evaporation at 20,000 yr BP than at present, our argument reaches the same conclusion from an independent line of reasoning.

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Glacial climates in the eastern tropical South Pacific

RECENT attempts to explain the glacial aridity at upper elevations in the Galapagos Islands have failed to take into account either concurrent events in neighbouring areas¹⁻⁶ or modern causes of climate. Yet, any reconstruction of the glacial climatology of the South Pacific must be consistent with all these data. Recognised glacial features of the region that must be taken into consideration and the empirical evidence which supports them include: increased upwelling along the equatorial divergence (enhanced plankton production⁷, simultaneous build up of guano⁸), upper elevation (650 m) aridity relative to the present in the Galapagos Islands (dated palaeomicrofloras⁹), increased precipitation above 2,000 m along the western slopes of the Peruvian Andes (deposits of oxides^{9,10}, traces of humid cycle stream cutting and scouring of alluvium⁹⁻¹¹, remains of sheet flooding at the base of the mountains^{9,10}, a disproportionate lowering of the west facing glacial snowline¹², relics of formerly more continuous plant and animal distributions^{13,14}), continuous aridity throughout the Pleistocene along the Peruvian coast (unbroken sequence of arid cycle processes⁹⁻¹¹), and exposure of large areas of the western Indo-Australian region following the 100-120 m sea level drop¹⁶ (fossil terraces^{16,17}). It can be deduced from the position of this exposed land that the modern pattern of vernal mixing of water from the eastern and western Pacific could not have taken place¹⁷ and that the eastern Pacific anticyclone did not, therefore, weaken in the glacial summer¹⁷. Thus, the pressure gradients between the two sides of the ocean would have remained fixed throughout the year and the eastern anticyclone and the south-east trade winds would have retained high intensity in both summer and winter.

At present, the low elevations of the Galapagos receive no moisture for most of the year and the upper elevations (1,000-1,700 m) are at best, covered by fog^{1,18,19}. The fog zone is the result of a temperature inversion brought about

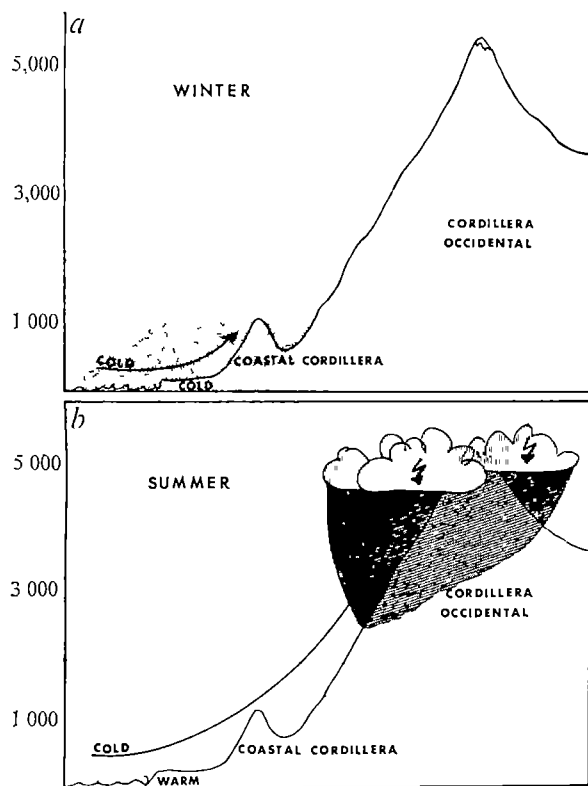


Fig 1 Schematic representation of modern day climate along the coast of Peru at approximately 12°S latitude (adapted from Bowman²²). *a*, Winter conditions with garua (fog) along the coast, and Andean summits clear, *b*, summer conditions with thundershowers and rain at high elevations and clear weather along the coast

by the cooling, compression and drag of the lower part of the air mass crossing the cold equatorial upwelling on its way to the Galapagos^{18,19}. Rains occur in the spring when the eastern Pacific pressure system weakens and permits the invasion of warm air masses from the north. This southern penetration of warm northern air constitutes the normal summer movement of the Intertropical Convergence Zone (ITCZ). An abnormally pronounced penetration and its accompanying storms is commonly called 'El Niño'^{20,21}.

In western Peru, modern rainfall patterns are also seasonal but are caused by semiannual changes in the temperatures of the land (t_l) relative to that of the bordering ocean (t_o). In the southern winter, onshore winds are very cold, having blown across the Peru Current and the coastal upwelling²⁰⁻²². These winds hit an equally cold or colder coast ($-3^\circ\text{C} < t_l - t_o < 1.5^\circ\text{C}$) lose their velocity and essentially stagnate in the form of the famous garua (fog) of the coastal hills²² (Fig 1a). Throughout this period, the high slopes of the Cordillera are sunny and dry. During the southern summer, the onshore winds are only slightly warmer than in winter (between 1° and 4°C , see refs 20, 21, 22) because they still cross the Peru Current and the coastal upwelling. The coast, however, heats up considerably (2° – 6°C) as the Sun moves south, resulting in a large land-ocean temperature difference ($1^\circ\text{C} < t_l - t_o < 6^\circ\text{C}$). The net effect of the cold onshore breezes hitting the warm coast is that the air is heated, absorbs energy and increases in velocity as it moves toward the nearby Andes. The strength with which the air hits the mountains triggers an unstable rapid rise. Condensation and thundershowers occur when the air reaches about 2,000 m (Fig 1b). In contrast to the fogs of winter, summer is clear along the coast.

The simplest model of glacial climatology should explain all of the ice-age features in the tropical South Pacific without requiring any *ad hoc* postulations of phenomena beyond those currently operative. Yet previous hypotheses

disregarded the implications for neighbouring areas or theorised major changes in the climatic pattern. Thus, the suggestion that the increase in moisture along the upper Andean slopes was caused by a weakening of the Peru Current^{9,10} or the coastal upwelling⁸ is unsubstantiated and overlooks the fact that decreases in the strengths of these currents would have allowed an invasion of warm water from the north and caused increased precipitation in the Galapagos and along coastal Peru. A similar hypothesis², that the glacial ITCZ remained south of the equator throughout the year, is inconsistent with arid conditions in the Galapagos and along the coast of Peru. The converse theory, that the ITCZ remained north of the equator during the year¹ does not seem likely on theoretical grounds³ and would have caused dry 'winter' conditions to prevail for almost 12 months along the high Andes. A recent analysis of correlations between surface sea temperature and the lack of rainfall in the Galapagos Islands led to the postulation that increased upwelling at the zone of equatorial divergence caused the aridity of the islands², but the study did not explain the causes for increased upwelling and similar reasoning was not extended to areas outside the zone of the divergence.

The model proposed here incorporates this most recent observation, accounts for all of the documented glacial events in the tropical South Pacific, allows for local climatic anomalies near upwellings and is based solely on substantiated changes in atmospheric conditions and modern patterns of climate.

Wind intensity and high atmospheric pressure were maintained throughout the year because of the prevention of the summer relaxation of the eastern Pacific anticyclone¹⁷ and the northward movement of the westerlies⁴⁻⁶ which resulted in compression of the south-east trade wind system. This year long maintenance of high atmospheric pressure and intense circulation led to correspondingly intense ocean currents and vigorous upwellings at the zone of equatorial divergence and along the Peruvian coast. Thus, waters as cold as, or colder than, those of the present winter were being brought to the surface or from higher latitudes near the Antarctic Ice Cap continuously throughout the year. Estimates from changes in carbonate composition and in the distributions of foraminifera^{23,24} indicate a cooling of ocean waters (5° – 6°C) off the coast of Peru. Estimates of low latitude, low elevation, land surface temperature depression are usually less than 3°C (ref 16). Along the Peruvian coast, unequal land-ocean cooling would have meant that temperature differences in a glacial winter would have approximated the present summer difference ($1^\circ\text{C} < t_l - t_o$). The modern summer pattern of arid lowlands and thundershowers at upper elevations, would have continued during most of the year. A corresponding increase in aridity at upper elevations in the Galapagos produced by lowered sea surface temperatures along the equatorial divergence was discussed by Houvenaghel².

It must be emphasised, however, that in the tropics, most climatic changes probably occurred near the beginning or the end of traditional temperate latitude glacial cycles, when ocean and land surface temperature differences were at a maximum and caused the most important effects of the Pleistocene in low latitudes—repetitive cycles of aridity and humidity¹³.

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Interaction of sodium dodecyl sulphate and polyethylene oxide

AN increase in the concentration of K_2CO_3 in Polyox WSR 205 causes a decrease in the drag reduction¹, this has been successfully correlated with lowered values of the intrinsic viscosity¹. Nonionic, water-soluble polypeptides can interact with sodium dodecyl sulphate (SDS) to form complexes², and above a certain concentration the reduced viscosity of some of these polymers is increased. The immediate implication is that if such interactions also take place with polyethylene oxides of high molecular weight then changes in the observed drag reduction may occur.

Some sort of soap-polymer interaction does occur (Fig 1), at all polymer concentrations the drag reduction is significantly improved in the surfactant solution. The turbulent flow line of SDS alone was not measurably different from that of pure water. This seems to be the first evidence that the drag reduction of a polymer in a good solvent can be slightly improved by the addition of a third component to the system. For example, at intermediate concentrations of 7-10 parts per million (p.p.m.) of Polyox WSR 205 there is a 35% increase in the observed drag reduction (Fig 1). The observed increase in drag reduction is similar to that which occurs at the lower Reynolds number of 6,000 (Fig 2). The increase in percent drag reduction over a similar concentration range is again of the order of 35%. The decreased effectiveness of the polymer-soap complex as the Reynolds number, Re , is increased is summarised in Table 1 where the intrinsic concentration, $[c]$, provides a measure of the efficiency of the drag reduction. It is obvious that the large increase in intrinsic concentration for the polymer-soap complex as compared with the polymer alone suggests that the complex

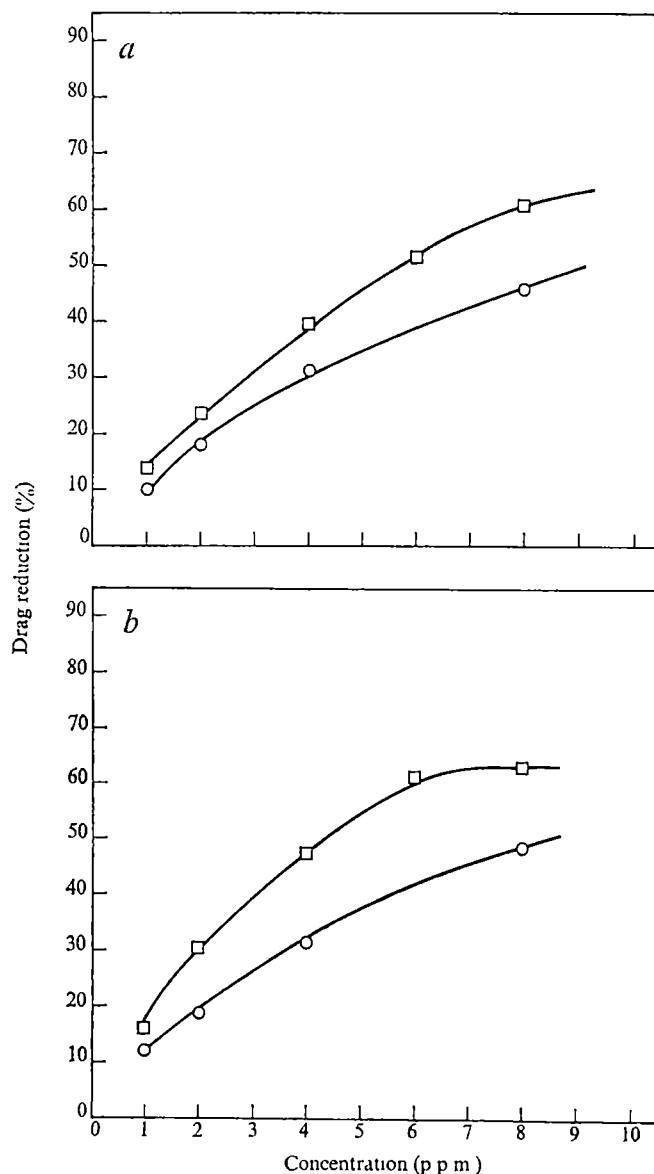


Fig. 1 Drag reduction against concentration for Polyox WSR 205 in water (O) and 0.2% SDS solutions (□) a, At $Re = 9,000$, b, at $Re = 6,000$

is breaking up at higher shear rates or stresses. This may not be too surprising in itself as aqueous drag reducing soap systems involving additional simple substances such as electrolytes³ and certain amines⁴ have exhibited stress-controlled drag reduction.

A ready explanation for this polymer-surfactant behaviour has been suggested². Apparently, in the present case hydrophobic bonding takes place between the polymer and the hydrocarbon tail of the surfactant. As sufficient surfactant becomes bonded to the polymer, electrostatic repulsion between bound surfactant ions on the polymer backbone causes the polymer coil to expand. Essentially, then, in the present case the equivalent of a polyelectrolyte is created through polymer-surfactant interaction. The dependence of such interactions on the nature of the surfactant and the polymer will be the subject of future work.

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Table 1 Effect of Reynolds number on complex

Solvent	$[c]$, $Re = 6,000$	$[c]$, $Re = 9,000$
H_2O	8.5	8.9
0.20% SDS	5.6	7.6

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Bond energy of the IO radical from molecular beam reactive scattering measurements

THE bond energy $D_0(\text{IO})$ of the IO radical is not reliably known. An early estimate, $D_0(\text{IO}) = 44 \text{ kcalorie mol}^{-1}$, was derived by Gaydon¹ from the IO emission spectrum of the CH_3I flame. Since this value was obtained by Birge-Sponer extrapolation of only five vibrational levels of the upper $\text{IO}(^2\Pi)$ state, it is not expected to be very accurate. The same spectrum was studied by Ramsay² in absorption by flash photolysis of O_2/I_2 mixtures. Birge-Sponer extrapolation of six bands gave $D_0(\text{IO}) = 42 \pm 5 \text{ kcalorie mol}^{-1}$. But more accurate extrapolation² for ClO and BrO using twenty bands showed that extrapolation from a small number of low lying levels leads to unreliable results. A rigorous upper bound $D_0(\text{IO}) < 62.8 \text{ kcalorie mol}^{-1}$ was determined from predissociation of the upper $\text{IO}(^2\Pi)$ state.

A value $D_0(\text{IO}) = 57 \pm 6 \text{ kcalorie mol}^{-1}$ was estimated by Sugden³ by photometry of the IO emission spectrum from a $\text{O}_2/\text{H}_2/\text{I}_2$ (1%) flame equilibrium. Two estimates^{4,5} have been made by extrapolation of the RKR potential curve of the upper $\text{IO}(^2\Pi)$ state with model potentials; one⁴ supporting the higher flame equilibrium value and the other⁵ supporting the lower Birge-Sponer value.

Recently, preliminary reports⁶⁻⁸ of O atom reactive scattering by ICl have shown an important reaction path to be



In our experiments, a crossed molecular beam apparatus⁹ with an electron bombardment mass spectrometer detector was used. The O atom beam was produced from a low pressure (~ 1 torr) microwave discharge through O_2 . The discharge region was separated from the beam source slit by ~ 60 cm of Pyrex tube. This configuration results in a low temperature ~ 350 K atom beam and suppresses $\text{O}_2(^1\Sigma_g^+)$ metastable molecules. The ICl cross beam issues from a supersonic nozzle source and has a narrow velocity distribution ($\sim 20\%$ full

width at half maximum). The most probable reactant translational energy was $E = 0.8 \text{ kcalorie mol}^{-1}$. The distributions of IO product velocities have been measured by a computer time-of-flight system over a range of laboratory scattering angles. Analysis of the full experimental data shows that the reaction proceeds by a long-lived complex mechanism.

The distribution of IO product translation energy in centre of mass coordinates is most accurately determined by observation of the IO velocity distribution along the direction of the centroid vector in laboratory coordinates. The IO flux density distribution measured at laboratory scattering angle $\Theta = 79^\circ$ (nominal centroid vector at $\Theta = 78^\circ$) is shown by open circles in Fig. 1. The distribution for the long-lived collision complex, predicted by a model¹⁰ based on RRKM theory of unimolecular decomposition, is also shown by a solid curve in Fig. 1. Agreement with the experimental data is obtained for a reaction exoergicity $\Delta D_0 = 3.4 \text{ kcalorie mol}^{-1}$. Using¹¹ $D_0(\text{ICl}) = 49.6 \text{ kcalorie mol}^{-1}$, we obtain $D_0(\text{IO}) = 53 \pm 3 \text{ kcalorie mol}^{-1}$.

This value of $D_0(\text{IO})$ also gives good agreement with our time of flight measurement of the $\text{O} + \text{I}_2 \rightarrow \text{IO} + \text{I}$ reaction. But the exoergicity, $\Delta D_0 = 17.4 \text{ kcalorie mol}^{-1}$, is greater for this reaction. Thus it does not provide as sensitive a test of $D_0(\text{IO})$ as the almost thermoneutral $\text{O} + \text{ICl}$ reaction.

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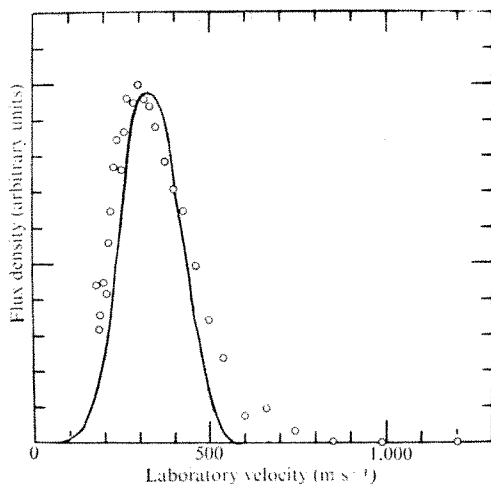


Fig. 1 Laboratory velocity distribution (flux density) of IO reaction product along the direction of the nominal centroid vector, $\text{O} + \text{ICl} \rightarrow \text{OI} + \text{Cl}$, $\Theta = 79^\circ$.

Unique form of filamentous carbon

CONSIDERABLE interest has been shown in the formation of carbon filaments from the catalytic decomposition of gases over metal surfaces at about 700°C (see refs 1, 2). Detailed studies have been chiefly confined to the behaviour of pure metals, but technically and industrially the effect of alloying elements is also important. We have studied how additives to an iron catalyst modify the filament growth process during decomposition of acetylene. With acetylene decomposed on a Pt/Fe alloy a new mode of filament growth was identified in which the complete detachment of a catalyst particle from the surface of the metal was not a necessary prerequisite for growth³. Here we describe the effect of tin on the growth of

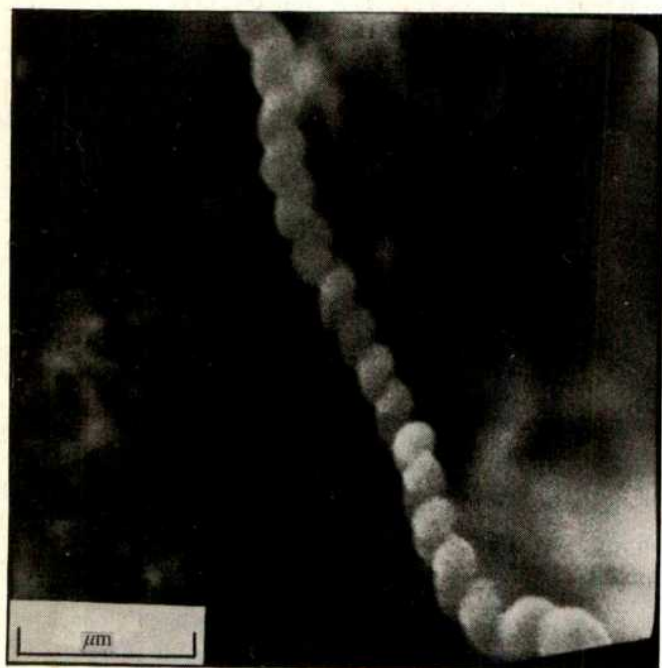


Fig. 1 Scanning micrograph of a spiral filament produced from Sn/Fe catalysed decomposition of 5 torr acetylene at 800°C.

filaments from the iron/acetylene system. The experimental technique used in this work, controlled atmosphere electron microscopy, has been described elsewhere⁴.

Significant deposit growth was only observed at temperatures above 800°C with tin coated iron foils in 5 torr acetylene and

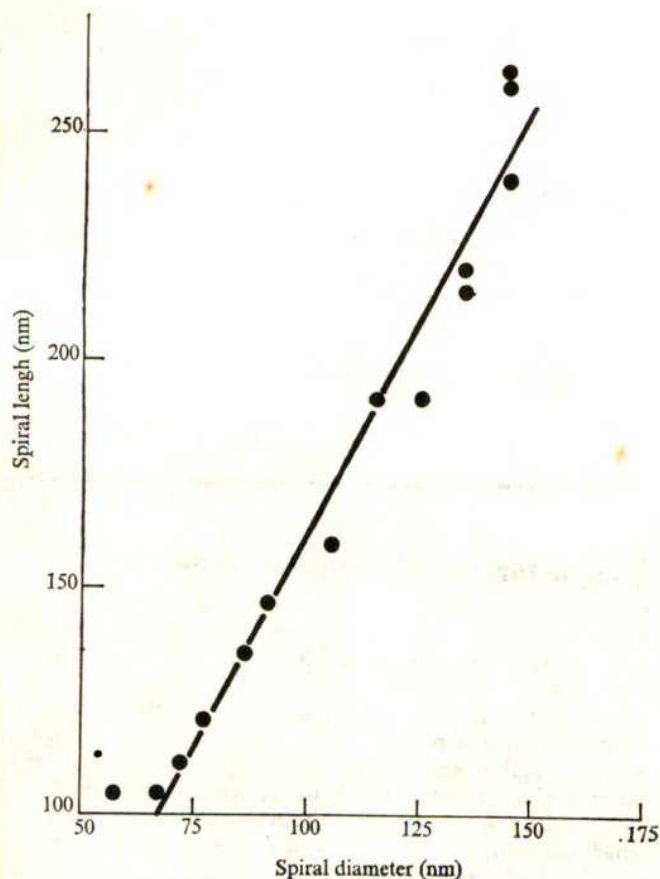


Fig. 2 Variation of spiral length with spiral diameter.

was preceded by an induction period of about 10 min at this temperature. On pure iron, deposits grew at temperatures as low as 650°C under otherwise similar conditions. Pure tin did not produce a deposit. In contrast to the growth on pure iron, which became autolytically poisoned after a few minutes, growth still continued on Sn/Fe after more than 2 h and poisoning was never observed. The deposit was filamentous, a common form of growth from acetylene^{1,2}, but the filaments were nearly all spirals with a constant pitch (Fig 1). Although spiral filaments have been observed in other systems^{1,5,6} they are rare and the production almost exclusively as spirals is unusual.

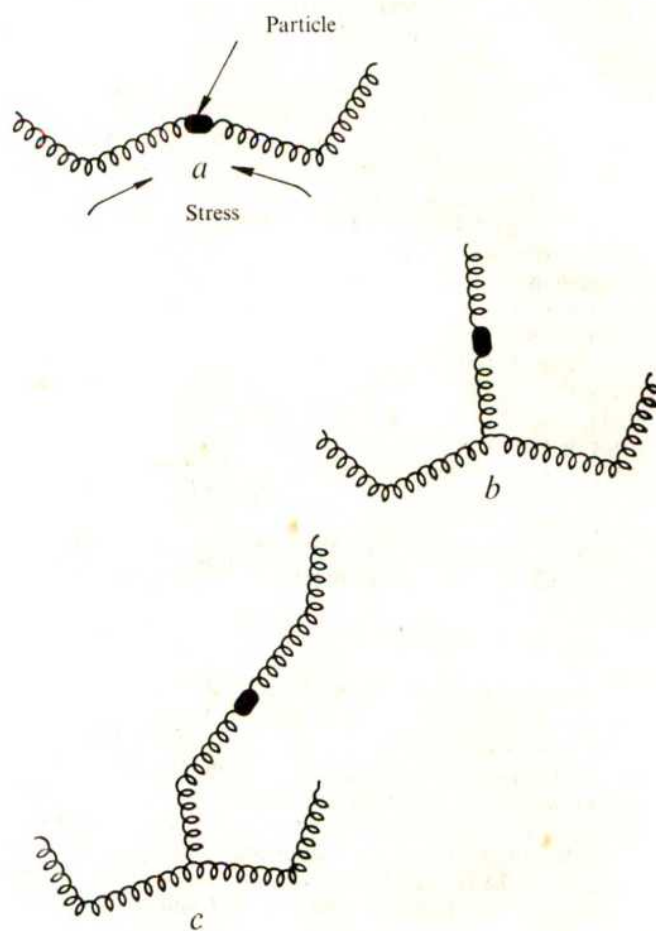


Fig. 3 Mode of formation of branched filaments.

The average pitch θ , can be obtained from a plot of the spiral length, l , against the diameter of the spiral, d ;

$$l = \pi d \tan \theta$$

and is found to be 30° 41' (Fig. 2).

Two filaments grew from each particle in opposite directions and it was not possible to discern the shape of the particle. The two filaments grew at identical rates and any kinks or imperfections in one were reproduced in the other so that the two filaments were exact enantiomorphs except for the handedness of the spirals which, viewed from the particle, were opposite handed in every case. Branching of filaments was common and seemed to be the principal means of relieving the stress created by the fact that filaments were often forced against the mass of deposit in two directions. When this stress became too great the particle was squeezed out from between these two

filaments and began to propagate a new pair, (Fig. 3). It is not possible at present to give a detailed account of the processes which constrain filaments to grow in this manner although it is certainly associated with the structural characteristics of the catalyst particle.

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Ancient boomerangs discovered in South Australia

DURING excavation in a South Australian peat quarry in January 1974, a wooden tool industry was found buried in basal peat formed between $10,200 \pm 150$ BP (ANU-1,292) and $8,990 \pm 120$ BP (ANU-1,293). Chert tools and chipping debris associated with swamp side encampment were also recovered from shoreline clays and underlying muds. Three implements associated with this industry are complete boomerangs (Fig. 1), suspected of being made from *Casuarina stricta* (Drooping Sheoak), a species growing above the swamp today. Although exact ages for the boomerangs are still to be determined, the finds provide the oldest evidence of the boomerang in the world and the collection as a whole is one of the most technologically complete in the Australian archaeological record. The collection of more than 25 wooden implements includes a simple short spear, at least two types of digging stick, and a barbed javelin fragment carved from a single piece of wood. Although several other implements were recovered complete, their functions are as yet unknown.

Featuring robust working edges made on large flake cores, typically worked into convex steep-edged scrapers, the stone tool industry conforms well to the 'Australian core-tool and scraper tradition' described for the 26,000-yr-old Lake Mungo industry^{1,2} and to which Pleistocene components at Keilor, Kenniff Cave, and Burrill Lake might also be assigned. Citing ethnographic examples and microscopic evidence, several researchers, particularly Jones³, have ascribed to these forms heavy wood-working tasks such as planing, cutting, debarking, and scraping—precisely the tasks Wyrie Swamp stone tools are believed to have performed. But, whereas food preparation/procurement roles are suspected of being present in the lithic technology, no direct dietary or faunal evidence was uncovered.

Current palaeobotanical studies of the peat deposits in the region made by John Dodson at the Australian National University (ANU) indicate trends in peat accumulation which appear to correlate with prehistoric utilisation of the bog. Principal Aboriginal visitation took place during an initial shallow water phase characterised by fluctuating wet, and occasional dry, episodes dated to between 10,200 and 9,000 yr ago. During this period, when the swamp was small, its shoreline became the scene of numerous tool-making and hunting and gathering activities centred on resources at the water's edge. However, 9,000 yr ago an increasingly more permanent water stand rose to cover most of the gently sloping bank, burying the shoreline debris and displacing tool-making on to the adjacent steep dune slopes. Evidence for subsequent use of the swamp appears

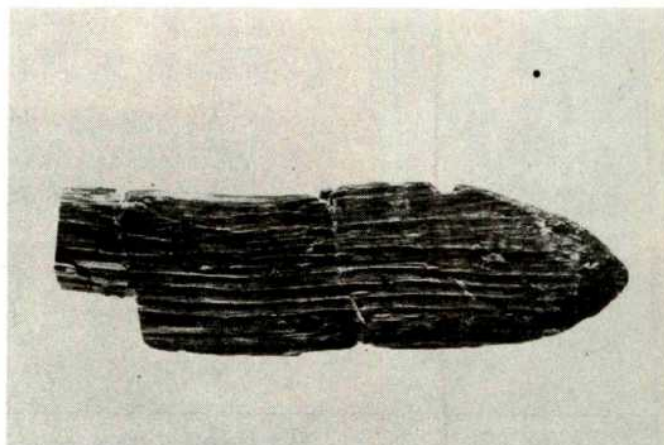


Fig. 1 This fragment of a fourth boomerang from Wyrie Swamp has been identified as *Casuarina stricta* root. Measuring 11 cm, it has been treated with polyethyleneglycol and freeze-dried.

infrequently until $7,930 \pm 160$ BP (ANU-1,377), possibly in response to the relocation of aquatic plant resources.

We can therefore see the Australian Aborigine emerging from the Pleistocene equipped with a tool kit as vital to the exploitation of the local environment then as it was yesterday, and just as complex. Exactly how long this technological tradition previously existed is as yet unknown but the possibility that the boomerang soared over the shores of Lake Mungo 16,000 yr earlier seems more plausible as a result of discoveries at Wyrie Swamp.

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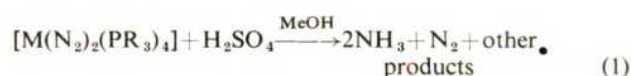
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The reduction of mono-coordinated molecular nitrogen to ammonia in a protic environment

WE have reduced ligating molecular nitrogen (dinitrogen) to ammonia in yields of up to 90% at a single metal site. This reaction is important for its possible application to our understanding of the chemical mechanism of the reduction of dinitrogen to ammonia by nitrogenase, where the reduction may occur at a single molybdenum ion site¹. Our reaction occurs when compounds of the type $[M(N_2)_2(PR_3)_4](I)$ ($M = Mo$ or W ; $R =$ alkyl or aryl) are treated at room temperature with sulphuric acid in methanol solution:



This reaction was performed in a vacuum so that the evolved gases could be analysed and measured. On mixing the reagents, one molecule of nitrogen gas was rapidly evolved with a trace of dihydrogen. The remaining dinitrogen was spontaneously

Table 1 Yields of ammonia and hydrazine from reaction of H_2SO_4 with tungsten and molybdenum complexes

Complex	Solvent	% NH_3^*	% N_2H_4^*	% N_2^\dagger
<i>cis</i> -[W(N ₂) ₂ (PMe ₂ Ph) ₄]	MeOH	90	2	94
	MeOH	88	2	92
	MeOH/H ₂ O‡	63	3	67
	THF	46	13	59
	THF	42	23	64
<i>trans</i> -[W(N ₂) ₂ (PMePh ₂) ₄]	THF	36	15	54
	THF§	36	< 1	34
<i>cis</i> -[Mo(N ₂) ₂ (PMe ₂ Ph) ₄]	MeOH	36	< 1	40
	MeOH	30	< 1	32
	THF	20	< 1	28
	THF	35	< 1	34
<i>trans</i> -[Mo(N ₂) ₂ (PMePh ₂) ₄]	THF	4	< 1	~ 5

*Percentage of $\text{N}_2 \rightarrow 2\text{NH}_3$ or $\text{N}_2 \rightarrow \text{N}_2\text{H}_4$; ammonia determined by indophenol reagent, hydrazine by *p*-dimethylaminobenzaldehyde reagent.

† Total N_2 regained by oxidation with NaOBr of all products of reaction after Kjeldahl distillation, identity of gas confirmed by mass spectroscopy.

‡ 1:1 mixture by volume, dinitrogen complex in suspension.

§ Run at about -2°C , all other reactions at 20°C .

reduced to ammonia together with some hydrazine, presumably also with concomitant oxidation of the metal M.

The highest yield of ammonia (90%) was obtained from *cis*-[W(N₂)₂(PMe₂Ph)₄] in methanol. In a non-protic solvent, tetrahydrofuran (THF), the yield of hydrazine was substantially increased, but that of ammonia was halved. In aqueous methanol, the yield of ammonia was also lower, possibly due to the lack of homogeneity in the reaction mixture. The compounds examined, the effect of solvents and yields obtained are listed Table 1.

The metal-containing products from the tungsten reactions were insoluble blue solids, but with molybdenum the reaction mixture remained as a yellow-brown solution throughout. These metal products also contained some nitrogen when the solvent was THF.

We have already shown² that the ditertiary phosphine complexes, *trans*-[M(N₂)₂(Ph₂PCH₂CH₂PPh₂)₂] (II; M = Mo or W), when treated with hydrogen bromide, quantitatively form complexes of diazene, HN=NH; however, the diazene ligand could not be protonated or reduced any further in a protic solvent, except perhaps in trace amounts. Sulphuric acid has similarly given a quantitative yield of hydrazido-(2-) complexes, tentatively formulated as [M(SO₄H)(N-NH₂) (Ph₂PCH₂CH₂PPh₂)₂] SO₄H on the basis of their infrared spectra and analysis; however, no further protonation occurred to give a significant quantity of ammonia or hydrazine. Very low yields of ammonia have also been claimed from the treatment of the complex (II; M=Mo) with iron-cluster compounds, for example, [Fe₄S₄(SEt)₄]³⁻ in THF followed by acid³.

The difference between the complexes (I) and (II) which allows the reduction to proceed in the former but not in the latter, is, we believe, the ability of (I) to lose a molecule of ligating tertiary phosphine. This would allow the attack of a second sulphate (or hydrogen sulphate) ion at the metal centre, increasing its electron density. The consequent flow of electronic charge into the N₂H₂ ligand would then trigger its further protonation and reduction to hydrazine and then to ammonia.

As far as the natural system is concerned, these reactions show that molecular nitrogen can be reduced at a single metal site in a protic medium with negligible discharge of dihydrogen or displacement of dinitrogen by hydride ligands. It may also be significant, in view of the special role of molybdenum in nitrogenase, that the reduction of mono-coordinated dinitrogen has been found for the first time in molybdenum and tungsten complexes.

The most commonly proposed mechanisms of reduction involve dimetal sites^{4,5}; and by protonation of dinitrogen-

bridged dimetal complexes, yields of ammonia and/or hydrazine of up to one dinitrogen molecule reduced for each dimetal unit have been obtained^{6,7}. Our system is the first observation of reduction, in a protic medium, of one molecule of dinitrogen per transition metal atom in a well defined complex. It shows that a monometal site is at least as likely as a dimetal site in nitrogenase.

In our reduction the metal must increase its oxidation state by six units ($\text{M}^0 \rightarrow \text{M}^{\text{VI}}$), all six electrons being fed from the metal into the ligating dinitrogen molecule. This great jump in oxidation state is highly improbable in any catalytic system such as that of the enzyme, but our reaction could provide a possible model for the feed of electrons from the metal atom into the dinitrogen molecule, on the atomic level, along the atom chain M-N-N. It also suggests that such feed of electrons and consequent reduction of dinitrogen, may be triggered by replacement of some 'soft' ligand on the molybdenum by a 'hard' one such as an oxygen ligand [for example, sulphate, phosphate, hydroxide or phenoxide (from tyrosine)]; the reverse replacement would occur during the metal reduction and dinitrogen pick-up stage of the enzyme action.

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Control of aquatic weed by moth larvae

AQUATIC weeds use up nutrients in the water and so reduce the capacity of cultured areas to produce fish^{1,2}. Chemical, manual and mechanical control methods can be too

expensive³ and biological weed control is ecologically acceptable and more dependable.

The Chinese grass carp (*Ctenopharyngodon idella* Val.) has proved efficient in controlling many common aquatic weeds⁴⁻⁸. Some insects too may destroy particular aquatic weeds⁹⁻¹³ but their use for biological control has not received much attention. Philipose¹⁴ found that certain moth larvae were able to feed on the leaves of *Pistia stratiotes* L., a common noxious floating weed, but did not identify them or describe the infestation.

Here we report that the larvae of the moth, *Erastroides curvifascia* Hampson, feed specifically on foliar and stem portions of *P. stratiotes* (which is not eaten by grass carp or any other phytophagous fish) and describe their use as a potential biological control agent for the weed in fishery waters.

Field observations were made in a perennial pond (0.35 ha) with a dense infestation of *Pistia*. The numbers of larvae are high during the winter months (that is November to February) (water temperature: 22–26° C) and therefore the consumption of the host plant is also extensive during this period. The entire life cycle of the moth is completed within 27–35 d. The larval stage, which is the active feeding phase, accounts for more than 50% of the life span. The late instar larva pupate in the deep furrows of leaves and petioles of *Pistia* after feeding for about 7–9 d. The pupae metamorphose into adults in about 5–6 d.

Three sets of preliminary trials were conducted in plastic pools (capacity 500 l; surface area 6,943 m²). Field conditions were simulated by providing 50 kg of pond soil at the bottom and 200 l of pond water in each set. The weed density in the three plastic pools was identical, each receiving 1,650 g of fresh *P. stratiotes*. Two different densities of the late instar larvae, 86 m⁻² and 17 m⁻² of the surface area, were tried in the first and second pools respectively, the third being the control. The larvae in the first pool cleared 1,340 g of weed in about 30 d, and 1,230 g of weed was cleared in 40 d in the second pool. The control set recorded an increment of 960 g in weed weight at the end of 40 d reflecting the growth of *Pistia* in the absence of predation by the moth larvae. It was observed that in both the experimental sets the larvae introduced initially pupated after a brief period of feeding, resulting in the emergence of moths which immediately bred. The new early instar larvae caused most of the damage to the plants.

Our study indicates the possibility of using the larvae of *E. curvifascia* for controlling *Pistia*, as they largely satisfy the basic requirements of a potential control agent. The comparative ease with which the larvae can be transferred *in situ* to host plants and the way the moths readily breed in the laboratory suggest a wide scope for disseminating this important agent for the control of *Pistia* in fishery waters.

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Hornet nest architecture

ALL social wasps belong to the family Vespidae. This family is divided into the following three sub-families: the Stenogastrinae—prevalent in South-east Asia, the Polistinae—of almost worldwide distribution, and the Vespinae—including the more socially developed forms which are encountered primarily in the Northern Hemisphere¹. The comb constructed by these wasps is fastened by a pedicle to the roof of the nest^{2,3}. In the course of the season the combs attain optimal size and acquire a spherical or elliptical shape, curving upwards at the centre, much like an inverted mushroom. The pedicles are long enough to allow clear passage of wasps from one comb to another.

Here I report observations and experiments carried out to elucidate the way in which hornets acquire the spatial perception necessary for the construction of combs with such clear-cut architectonic lines.

Hornets (3 d old) were maintained in groups of 30–40 individuals in specially constructed wooden breeding boxes with one wall of transparent glass⁴. Observations were made on the comb-building activities of intact hornets as compared with those of hornets with variously amputated wings. The wings were treated in one of four ways: first, one pair of wings was amputated; second, both pairs of wings were amputated; third, same as the first but subsequent maintenance of the hornets for 3–4 d at a temperature of 8° C; fourth, same as the first but with supplementation of the diet with queen pheromones (C₁₆ lactose).

The standard diet in all the experimental breeding boxes was 30% sucrose solution *ad libitum* as well as daily quotas of wasp or bee pupae. Clumps of clay soil were introduced as building material for the wasps. All experiments were carried out in a vesparium whose dimensions are given elsewhere⁵ and in which the temperature was kept at 27–28° C, as optimal for hornet activity⁶, while the relative humidity was 65–70%. Each experiment was repeated ten times.

Hornets with intact wings, after several days in the breeding box, commence building pedicles which are suspended from the roof of the box and serve as foundations for combs that architectonically resemble those produced in nature. The combs are constructed one next to the other and in parallel to the roof. The initial combs are small, each consisting of 20–30 cells, but ultimately they merge to form a single, large comb the breadth of which is functional to and only slightly smaller than the breadth of the roof. At the lateral margins of the comb there are always narrow gaps between it and the frame of the breeding box which permit movement of hornets to and from the comb. Similarly at the top of the comb, because of the pedicles which suspend the comb from the roof of the breeding box, there is a narrow space which enables passage of the worker or queen hornets as in nature. The width of this gap is 1.5–1.7 cm.

Hornets which had one pair of wings amputated also constructed a number of combs, but their construction differed in the following respects. The combs were fastened by pedicles to the wall of the breeding box rather than to its roof, that is at right angles to the norm. They were built one beneath the other, rather than one beside the other; that is, along a vertical axis rather than in the customary horizontal placement. The vertical distance between two adjacent combs was about twice that in normal or natural construction (3.2–3.4 cm). The combs do not merge but

remain discrete and small, consisting of about 50–60 cells each (Fig. 1, lower three combs). The pedicle, which in the normal comb was a compact rod narrow at the middle and wide at both ends was in this case merely a single or double row of 2–4 cells which formed a narrow bridge between the wall of the box and the comb (Fig. 1). Oviposition into the cells which substitute for a pedicle never occurred, but the workers did oviposit into other comb cells and subsequently nursed the emerging all-male brood. Structurally, the single comb of wing-amputated hornets resembled a mirror half-image of a normal complete comb.

Hornets with both pairs of wings amputated constructed their combs as described before but without a pedicle, that is, the combs were constructed right against the wall of the breeding box. Cooling or pheromone-feeding of the wing-amputated hornets caused them to revert to normal comb construction. It is worth mentioning that both hornets with

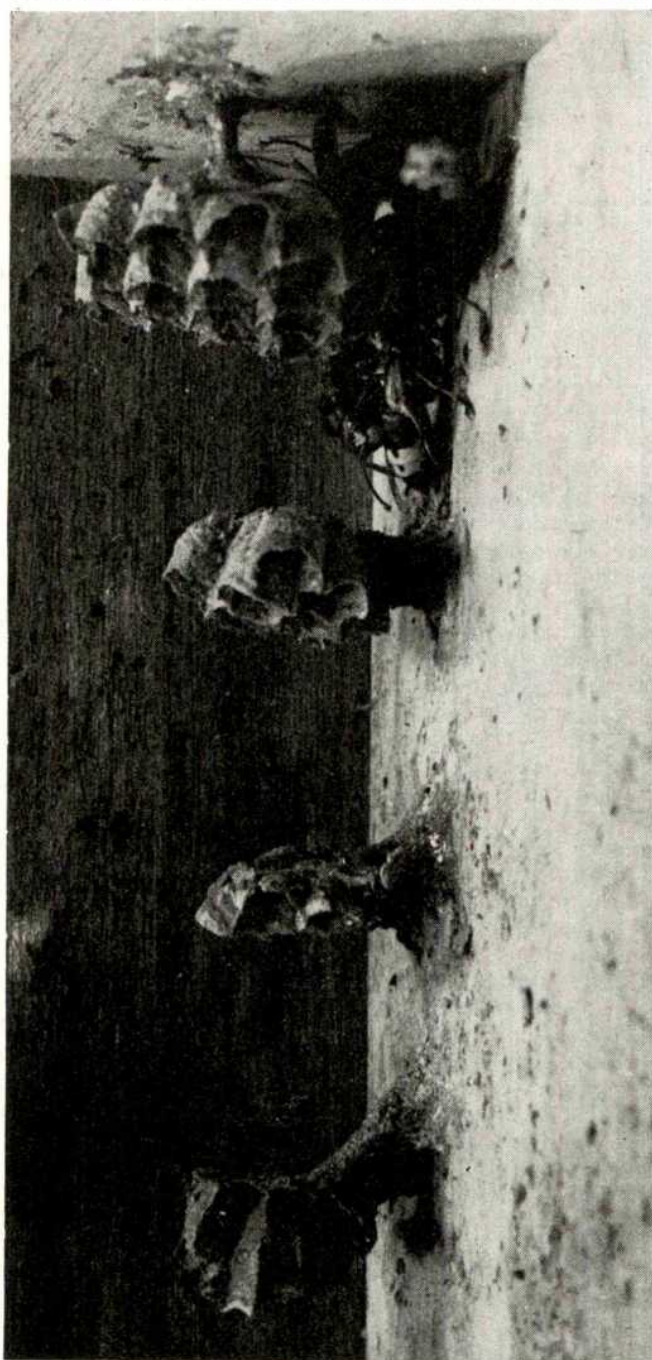


Fig. 1 Lateral view of hornet combs. The topmost comb represents normal construction. The three bottom combs were constructed by hornets who had one pair of wings amputated. The pedicle in these combs is along the same plane as the comb itself rather than vertical to it.

intact or amputated wings built cells of the same size and invariably the cell apertures faced downwards.

I believe that the vertical distance between two adjacent combs constructed by wing-amputated hornets should be regarded as analogous (at a 90° displacement) to the horizontal distance between two adjacent combs constructed by hornets with intact wings. The results clearly indicate that comb construction by wing-amputated hornets is different from that by normal, intact hornets. The most prominent deviation in comb construction is the different spatial orientation of the combs, which suggests that the wing tips of hornets figure importantly in the spatial perception. Amputation of the wings seemingly causes a displacement of the sensory feedback of the spatial perception and thus apparently results in deviations or errors in the orientation of construction, in incorrect distances between adjacent combs and in unilateral comb architecture. A constructing hornet, while moving about at the construction site, acquires an awareness of its spatial orientation. Von Holst⁷ designated this awareness refference, implying such impulses arise because of movement of the organism. I have no reasonable explanation as to how pheromones induce habituation of the anatomical displacement in wing-amputated hornets, but it is possible that the cooling for several days retards or prevents the ripening of their ovaries and thus inhibits the building instinct without, however, curtailing their movements. Thus the moving hornet, in its unfamiliar asymmetric situation, is able to acquire the feeling of refference and within a few days learns to orient itself to the new spatial conditions by readjustment of the sensorial feedback.

In Vespinae, the tips of the wings of the workers usually extend close to or even slightly past the tip of the abdomen. In contrast, in Stenogastrinae, the wings are always relatively short, covering only the anterior segments of the abdomen. The combs of the latter vespids are comprised of a relatively small number of cells⁸ and lack a pedicle, being fastened directly to the substrate⁹ resembling those built by hornets with two pairs of wings amputated.

It is possible that amputation of the wings of Vespinae, as I have done with *Vespa orientalis* converts the spatial perception of these hornets to that commonly displayed by Stenogastrinae. There may also be a connection between the architectural building in the Polybiine (Polistinae) wasps—phragmocytarous as against stelocytarous—and the length of their wings.

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Extraretinal control of vertical migration in fish larvae

MANY species of fish, including the Atlantic herring *Clupea harengus* and its larvae, undergo diel vertical migrations^{1,2}. In the herring and plaice larvae this migration has been shown

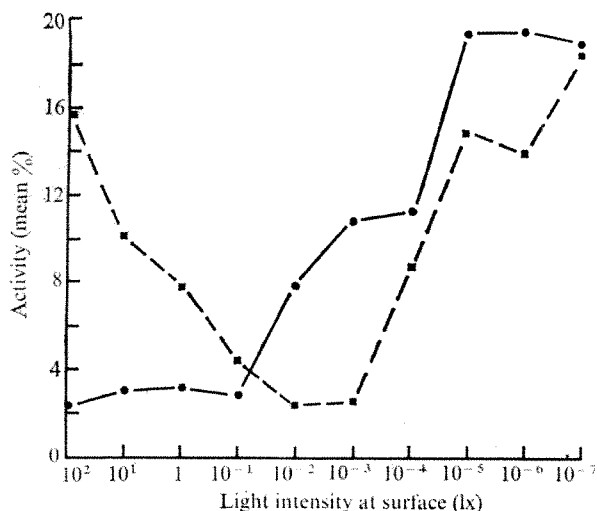


Fig. 1 The change in surface activity of normal and eyeless herring larvae in response to a reduction in ambient light intensity. Groups of 20 larvae were placed in seawater in an acrylic column (internal diameter 5.1 cm, water depth 117 cm) located in a light-tight box and illuminated from above by a 12 V 40 W tungsten lamp run at 12 V giving a light intensity of 10^2 lx at the water surface. The light beam passed through three glass heat filters then through one of a series of neutral density filters. The neutral density filters were located in a wheel which changed automatically every 15 min reducing the light by a factor of 10 to a final level of 10^{-9} times the original light intensity. A pair of matched thermistors in a balanced bridge circuit were inserted in the water column, 2 cm below the water surface, to record larval activity. Activity thus recorded has been shown to give an estimation of the number of larvae active at that level³. Larval movements detected by the cooling of the thermistors were recorded on paper for later analysis (activity only assessed during latter 10 min of exposure at each intensity). Herring and plaice larvae, both with eyes and in an eyeless condition, were tested in this apparatus. The eyes were removed surgically from anaesthetised larvae at a variety of developmental stages and as far as possible each group of experimental larvae was at the same stage. Within the range of larval stages tested the responses of the larvae were found to be comparable and the results have been pooled. The activity has been plotted as a percentage of the total activity to compensate for differences in activity level between experimental groups. The light intensity was measured with an EEL Microphotometer with a maximum sensitivity between 450 and 550 nm, the peak being about 510 nm. ●, Blind ($n = 10$); ■, controls ($n = 5$).

to be evoked by changes in light intensity³. Under laboratory conditions they move towards the water surface at dusk and return down the water column at dawn when exposed to natural light. Moreover this vertical migration can be evoked at any time of day by reducing, then increasing, the ambient light intensity³. The control of this behaviour is shown here to be at least partly mediated through extraretinal photoreceptors in the larvae of herring and plaice, *Pleuronectes platessa*.

Figure 1 clearly shows that the eyeless larvae respond to a reduction in light intensity greater than a factor of 10^{-3} by moving towards the water surface in a similar fashion to the control larvae. Also the activity, and hence the number of larvae at the surface, increases as the light intensity is further decreased. Although the activity patterns of eyeless and control larvae are not identical, they are similar enough to suggest that the same extraretinal photosense may be responsible in both conditions. Figure 2 shows that returning the light to its original intensity rapidly abolishes the surface activity of eyeless larvae, thus reversing the direction of migration.

At higher light intensities (Fig. 1) the normal larvae exhibit a high surface activity whereas the eyeless larvae remain lower in the water column. This initial response of the control animals appears to be phototactic. Blaxter⁴ has shown herring to be positively or negatively phototactic depending on the light intensity; and the range of intensities, over which this initial surface activity occurs, corresponds approximately to

that at which he observed positive phototaxis. Eyeless larvae are not phototactic over the range of light intensities 10^2 to 10^{-7} lx. Plaice larvae respond in a less marked but similar manner to herring larvae.

This correlation of the vertical migration of eyeless and control larvae is particularly interesting as it indicates that extraretinal photosensitivity plays an important role in the daily regulation of the behaviour of normal larvae. Moreover, the activity of the larvae, particularly at intermediate light intensities is influenced by the input of both the eyes and extraretinal photoreceptors. The retinal and extraretinal photosenses therefore seem to interact in producing the observed behaviour.

A suggestion that such interaction of photosenses may occur in adult fish may be seen in the work of Jones⁵. He demonstrated that minnows, *Phoxinus phoxinus*, are less active by day than by night when blind, whereas eyed fish in the absence of cover show the opposite pattern of activity. Where adequate cover is provided, however, the daytime activity of the eyed fish is considerably reduced and greater activity is displayed at night, especially observed at dawn and dusk. From this it can clearly be inferred that, as with the herring larvae, the observed behaviour results from retinally and extraretinally evoked behaviour.

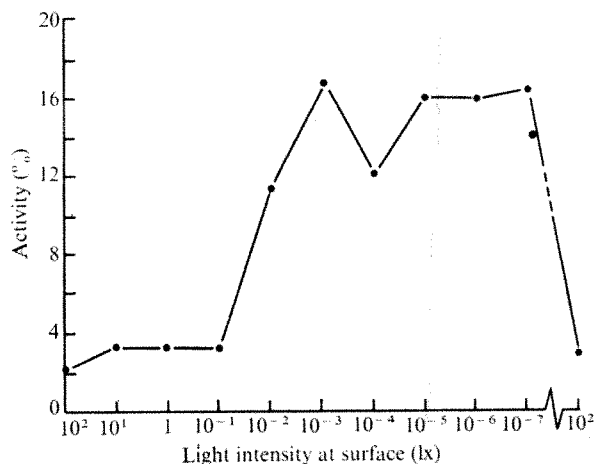


Fig. 2 The response of a single group of eyeless larvae showing the rapid manner in which surface activity is decreased when the light is returned to its original level. The interval between the increase in light intensity and activity measurement was 20 min.

It would seem that the light intensity dependent behaviour in these two examples could be divided into retinally evoked phototaxis and an extraretinally evoked kinesis which cannot be separated in sighted larvae. Further work is in progress to study the interaction of the two photosenses in modulating the animal's behaviour. Nothing can be said at this stage of the location of this extraretinal photosense but attention is drawn to a recent paper on trout⁶ which showed their diel activity pattern to be relatively unaltered by the removal of both the eyes and the pineal gland.

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Effect of absence of cochlear outer hair cells on behavioural auditory threshold

THE function of the two populations of sensory cells in the mammalian inner ear is not well understood. Anatomical evidence indicates that the inner hair cells (IHCs) and the outer hair cells (OHCs) play separate roles in the transduction of acoustic stimuli¹. Furthermore, there have been numerous proposals attributing different roles to the two hair cell populations in the production of the various cochlear potentials²⁻⁵. On the other hand, theoretical considerations and the interpretation of data from several experiments have led to suggestions of different types of interaction between the OHCs and IHCs⁶⁻⁸.

A common means of investigating the relative roles of the two groups of sensory cells is to destroy portions of one population, usually the OHCs, and assess the changes thus produced in various indices of cochlear function. A valuable tool in such studies has been the application of aminoglycoside antibiotics. These agents are specifically toxic to cochlear hair cells, OHCs being more vulnerable than IHCs at some cochlear locations^{9,10}. Independent measurements of OHC and IHC function have been obtained by treating animals with these antibiotics, thereby largely destroying the OHC population in parts of the cochlea, while leaving much of the IHC population intact. Electrophysiological recordings from the cochlea or the VIIIth nerve were then collected and compared with responses obtained from normal inner ears³⁻⁵.

The present study was undertaken to examine the function of the OHC and IHC more directly, by measuring the behavioural pure tone thresholds of animals treated with kanamycin. We were particularly interested in determining whether or not a characteristic audiometric pattern could be related to the selective destruction of portions of the OHC population.

Eight adult male chinchillas were first trained in a shock avoidance task similar to that developed by Miller¹¹. The shuttle-box, located in a small anechoic chamber, was calibrated at 54 points spaced equally about the cage. Results were averaged to determine sound pressure levels. Stable threshold curves were usually obtained within 3-5 d after the initiation of training. One threshold curve was collected in one session per day for each animal. Ten such curves were obtained before the commencement of kanamycin treatment, and averaged to produce a baseline measure of sensitivity from 100 Hz to 16 kHz. Normal thresholds were similar to those obtained by Miller¹¹.

The animals were injected subcutaneously with kanamycin (200 mg kg⁻¹ d⁻¹). This dosage was increased to 300 mg kg⁻¹ in two animals which were resistant to the drug. Daily threshold determinations were carried out and treatment was terminated when a significant shift in threshold was observed. When the threshold shift seemed to have stabilised, ten threshold functions were again obtained and averaged to produce a post-treatment measure of sensitivity.

Animals were then anaesthetised with urethane and paired, intracochlear electrodes were implanted in scala vestibuli and scala tympani of the basal turn, following which cochlear microphonic (CM) data were collected. Finally, both cochleae were perfused with osmic acid and prepared for histological examination either by the surface preparation¹² or the Araldite embedding technique¹³.

Initial shifts in threshold occurred at the highest frequencies, appearing after 6-35 d of kanamycin treatment. The increase in threshold spread towards the lower frequencies and stabilised within 7-15 d after termination of injections. One animal became totally deaf, within the limits of our equipment to determine, but died without electrophysiological or histological data being obtained. Two animals with relatively severe hearing loss became ill and were killed without collecting electrophysiological data. The remaining five animals survived from 3 to 5 weeks after treatment was terminated, before being killed. One

animal which suffered considerable IHC damage is not included in the discussion below.

Figure 1a and b shows the OHC counts and the shifts in behavioural threshold from six animals, respectively, arranged according to the severity of hair cell loss. Note that the behavioural manifestation of OHC loss in the basal region of the cochlea was a threshold shift at high frequencies; thresholds at low frequencies remained virtually unaffected. The agreement between the extent of OHC loss and the frequency region of threshold shift is quite good. To assess this agreement, the distance from the apical end of the cochlea at which 50% OHC loss occurred, and the lowest frequency at which normal thresholds were obtained, gave a correlation (Spearman Rank Order) coefficient $r = 0.86$.

Figure 2 shows the behavioural and histological results from one animal. IHCs were present throughout the cochlea and the OHCs disappeared between 11 and 13 mm from the apex. At the higher frequencies, the threshold shift ranged from 42 to 52 dB. In the five animals surviving drug treatment, thresholds were measured at 21 shifted frequencies which were well above the transition region. The amount of shift ranged from 30.2 to 52.3 dB (mean 39.9 dB).

To ensure that the high frequency shifted thresholds appropriately reflected the response of IHCs alone, it was necessary to show that the high frequency responses were not somehow produced by the low frequency, undamaged portion of the cochlea. A masking experiment was therefore performed. White noise, low-pass filtered with a rolloff of 84 dB per octave at 3 kHz, was introduced continuously into the test environment. A noise level was chosen such that thresholds at 3 kHz and all lower frequencies were increased by about 40 dB. Figure 2b shows that the high frequency thresholds were not affected, clearly indicating that they were produced in the unmasked region of the cochlea and thus they truly reflect the sensitivity of the IHCs in the absence of OHCs. CM rolloff matched the behaviourally measured loss of sensitivity in all animals for which it was measured.

Our interpretation of these data depends on two major assumptions. First, we assume that the IHCs that appeared intact under the phase contrast microscope were functionally

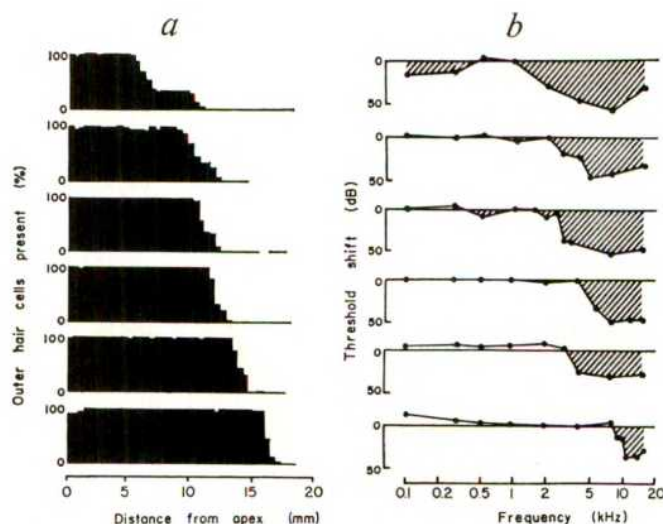


Fig. 1 a, Distribution of OHCs present in six animals, all three rows combined. Region counted is indicated by extent of the baseline. Top animal died during treatment. Ordinate, percentage of OHCs present at a given cochlear location; abscissa, distance along the basilar membrane, measured from the apical end. In all animals the IHC population (range 95-100%) was virtually intact. b, Threshold shift in the same six animals (hatching). Plots represent difference between ten audiograms obtained and averaged before and after drug treatment, except for the top animal. In the latter case, the audiogram obtained on the last day before death was used as the post-treatment measure.

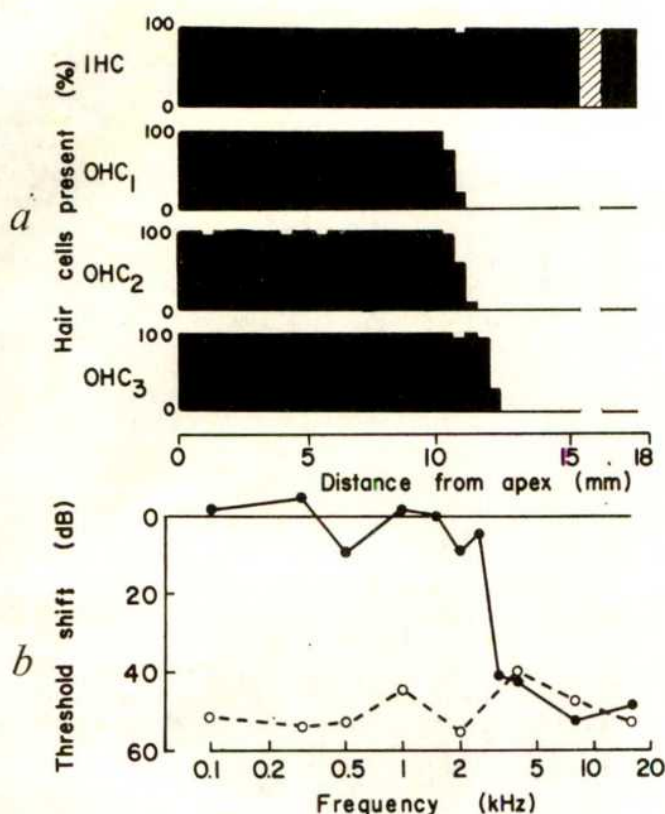


Fig. 2 *a*, Distribution of hair cells present along the cochlea in one animal. Subscript indicates row of OHCs. Hatching in the IHC plot, and gaps in the OHC baselines, represent a region of the cochlea which cannot be counted. *b*, Threshold shift measured after kanamycin treatment (●). Threshold shift measured after kanamycin treatment with low-pass (<3 kHz) masking noise present (○).

normal¹⁴. (Subtle morphological changes in hair cells, appearing normal when examined under the light microscope, may be observed with the electron microscope¹⁴.) Whether such changes influence hair cell function is open to speculation. Our working hypothesis is that subtle changes in cytoarchitecture would not significantly impair the transducer properties of the cell. While this cannot be directly confirmed, the fact that behavioural thresholds were completely normal at all frequencies below the region of threshold shift provides a basis for making this assumption. Secondly, we are assuming that the observed threshold changes are directly related to hair cell losses; that is, we assume that the drug treatment did not affect neurones of the afferent VIIIth nerve. Given the good agreement between the behavioural, physiological and histological data, this seems reasonable.

These data have several, potentially important implications. Firstly, it is apparent that in direct contrast with many studies using intense sound to create hair cell damage¹⁵⁻¹⁸, kanamycin ototoxicity yields such cochlear lesions that histological and audiometric measures are well correlated. In other words, with the well defined damage patterns observed, there is no sign of anomalous findings such as normal threshold in the presence of significant hair cell loss, or threshold shift in the absence of hair cell loss. Our data clearly show that in the chinchilla, just as in the guinea pig^{9,10}, kanamycin poisoning induces a selective OHC loss that progresses from base to apex. This permits the fashioning of cochleae in which the basal half is completely denuded of OHCs without significant IHC loss. Clearly, when one wishes to study IHC function, either with behavioural or physiological means, preparations of this type are greatly preferable to those where mixed hair cell losses tend to occur, resulting in a patchy damage pattern.

It seems therefore that the loss of OHCs has a well defined effect on auditory sensitivity. Audiometric thresholds are

about 40 dB poorer at frequencies where OHCs are absent from the corresponding cochlear location. This shift in sensitivity is extremely well correlated with the finding that the threshold change of the whole nerve action potential (AP) in guinea pigs having similar cochlear lesions is also of the order of 40 dB (ref. 5). We have also noted previously that the difference in cochlear microphonic production between OHCs and IHCs is between 30 and 40 dB (ref. 5). Thus a good three-way correlation among the various indices of cochlear function can be seen, all indicating that OHCs are the primary determinants of low intensity auditory behaviour, or at least of sensitivity. These findings are of considerable theoretical importance when one considers Spoendlin's¹⁹ demonstration that about 95% of the afferent auditory nerve fibres innervate the inner hair cells, and when the apparent homogeneity of response characteristics of auditory nerve fibres²⁰ is also taken into account. It seems probable that there is interaction between the outputs of OHCs and IHCs, either synaptic or ephaptic. Although there is considerable controversy over the existence of sufficiently close apposition (or synaptic contacts) between outer and inner hair cell dendrites to permit significant interaction^{1,21}, the experimental results seem to suggest such. These interactions do not appear to be of such nature that OHCs would inhibit IHC output as has been suggested⁸. Instead, the most parsimonious assumption is that the OHCs are specialised to perform a facilitatory function on the IHCs. The homogeneity of neural recordings suggest that all such data are probably obtained from a single population, most likely from the more numerous fibres which originate on IHCs. Behavioural and peripheral physiological data suggest that without OHCs the system is incapable of operating at low sound levels. These considerations lead to the conclusion that information processing is performed by the IHCs but that, at least at low intensities, they require facilitation by the more sensitive OHCs. Complex interactions between OHCs and IHCs have been suggested by several authors. We think that the interaction is probably not more complex than the above postulated facilitation. To demonstrate this, extensive behavioural data are necessary, collected in the absence of OHCs, on suprathreshold auditory properties and on frequency discrimination. Such data are now being obtained in our laboratory.

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Enrichment of fungal mutants by selective cell-wall lysis

SEVERAL procedures for enrichment of fungal mutants are known and have been reviewed^{1,2} but they have many limitations. Here we describe a new technique based on selective cell-wall lysis, which has wide applicability and is highly efficient for isolating mutants differing from the wild-type strain in growth requirements.

From the time of the early basic publications³⁻⁷, emphasis has been put on the importance of the physiological age of cells as regards protoplast formation by wall-lytic enzymes. Young cells are much more apt to yield protoplasts than are old ones.

Our method makes use of this fact and conditions are employed in which only the parental cells and not the desired mutants, can grow. The parental cells then become physiologically 'young' and sensitive to wall-degrading enzymes, whilst the mutants remain resistant, and can therefore be selected.

If the enzyme treatment is carried out in an osmotically-stabilised medium, the sensitive cells give rise to protoplasts which may be lysed by diluting the medium. This selective killing may also be achieved directly by using an environment not stabilised osmotically. The required mutants can then be isolated on selective media.

Here we report results obtained primarily with *Schizosaccharomyces pombe* wild-type strain L972 *h*⁻, with the adenine-requiring strain⁸ M210 *ade6 h*⁻, and with a number of auxotrophic and temperature-sensitive mutants of the wild-type strain. If not otherwise stated, cells were cultivated in liquid minimal medium containing vitamins⁷ at pH 5.5 with or without additional nutrients. The cell wall was degraded with a sterile-filtered, freeze-dried preparation of the digestive juice of the snail *Helix pomatia*. A 2% (w/v) solution of the digestive juice was prepared in this culture medium, without the use of an osmotic stabiliser. Enzyme treatment was performed with shaking at 25°C and a cell concentration of 10⁵ ml⁻¹.

To establish the optimum treatment time, suspensions in minimal medium were prepared from young and 1-week old cells of the wild-type strain. Snail enzyme preparation was added to the cells and samples were taken at intervals. Through the action of the enzyme preparation the young cells began to yield protoplasts, which immediately underwent lysis in the unstabilised medium. The rate of cell-wall degradation was followed indirectly by determination of the number of surviving cells. The data are plotted in Fig. 1a. After about 3 h practically all of the young cells had been killed, whereas the number of old cells had barely changed. On longer treatment, however, the cell-wall degradation of these also became apparent.

The behaviour of young and old adenine-requiring cells agreed with that of corresponding wild-type cells.

In the model enrichment experiments we used the wild-type strain and also the adenine-requiring mutant, which forms

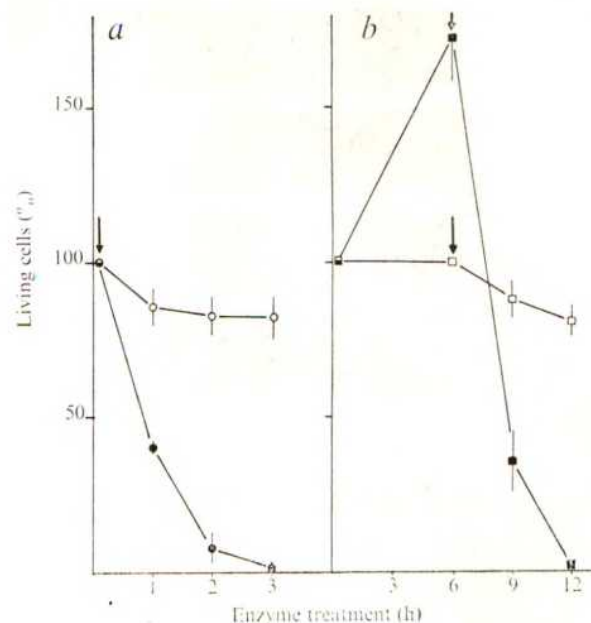


Fig. 1 Effects of enzyme treatment on *Schizosaccharomyces pombe* cells. a, Surviving cells of wild-type young (●) and old (○) cultures; b, surviving cells of wild-type (■) and adenine-requiring (□) cultures after 6 h incubation in minimal medium and 6 h enzyme treatment. Arrows indicate the addition of snail enzyme. Bars represent standard errors.

red colonies on media of low adenine content. As the colonies of the wild-type strain are white, the two types of colony can easily be distinguished. For better approximation to reality, a 100:1 mixture of wild-type and auxotrophic cells from 4-d-old colonies was used. The enzyme treatment was begun after an incubation of 6 h in minimal medium allowing exponential growth for the wild-type strain but not for the auxotrophic mutant. Figure 1b shows that after a 6 h enzyme treatment the wild-type cells were nearly all killed, while the majority of the mutant cells remained.

The efficiency of selective cell-wall lysis was enhanced markedly with the use of 2-deoxyglucose, an inhibitor of cell-wall synthesis⁹⁻¹¹. Work involving 2-deoxyglucose will be reported elsewhere. The following experiments, aimed at the isolation of new mutants, took place without 2-deoxyglucose.

In the actual enrichment process, cells of the wild-type strain, spread on the surface of yeast-extract-glucose agar medium, were subjected to ultraviolet irradiation from a Philips TUV 15 W lamp (450 erg mm⁻²; 1% survival). After subsequent incubation for 4 d, during which the mutations were manifested and the cells became old as regards cell-wall lysis, the colonies formed from the surviving cells were washed off, diluted to 10⁵ cells ml⁻¹ with minimal medium and incubated for 6 h at 25°C. This was followed by enzyme treatment for 6 h. Samples were taken before and at the end of the enzyme treatment. The samples were spread on yeast-extract-glucose agar medium, incubated for 2 d, and the colonies appearing were then replicated on to minimal medium. The mutants were isolated and their requirements determined. The degree of enrichment was found by comparison of samples taken before and after enzyme treatment.

The average values are based on data obtained with 18 different mutants, with which the ratios could be calculated exactly. In these cases the average proportion of mutants in non-enriched samples was 0.57±0.07%. The enrichment calculated on the basis of the average of the individual enrichments was 79.7-fold (*s*_x = ±14.4). No correlation could be found between the type of auxotrophic mutant and the proportion of enrichment. In many experiments it was not possible to establish the exact value of the enrichment, for the mutant could not be detected in samples which were not enriched.

Temperature-sensitive mutants from the wild-type strain have also been isolated by using ultraviolet irradiation and the enrichment procedure described above. Those mutants were isolated which could readily grow at 25°C, but for which 35°C was non-permissive. In this case an average enrichment of 80.5-fold ($s_x = \pm 17.2$) was obtained, higher even than for the nutritionally-deficient mutants.

The results can be still further improved, with correspondingly good efficiency, by repetition of the enrichment procedure.

Selective cell-wall lysis similar to that described here for *Schiz pombe* was also attained with snail enzyme or enzymes of microbial origin for *Saccharomyces cerevisiae*, *Candida albicans*, *Geotrichum candidum*, *Rhodotorula mucilaginosa* and *Aspergillus nidulans*. Frequently, however, supplementary procedures were also necessary to achieve efficient cell-wall degradation. For *S. cerevisiae*, *C. albicans* and *G. candidum* the effect of snail enzyme was optimum in the presence of 0.1% 2-mercaptoethanol. Selective lysis could be induced in all cases with cell-wall degrading enzymes of certain *Streptomyces* and *Actinomyces*. The details will be reported elsewhere.

Protoplasts have already been produced from cells of a large number of fungal species by means of snail digestive juice or enzymes of microorganisms. Thus, the proposed enrichment method based on selective cell-wall degradation may be widely applicable to fungal mutants differing from the wild-type strain in growth requirements, and possibly to mutant cells of higher plants in cell culture.

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Activity of vitamin A analogues in cell cultures of mouse epidermis and organ cultures of hamster trachea

FIFTY years after the discovery that vitamin A controls cell differentiation in epithelial tissues the mechanism involved is still unknown. The recent introduction of two-cell and organ culture systems, involving serum-free medium, for the assay of vitamin A activity *in vitro* may help. One assay uses epidermal cell cultures derived from mouse skin¹ and the other uses tracheal organ cultures from vitamin A-deficient hamsters². In both, the observed cellular response depends on the addition of vitamin A, which induces a marked increase in the cellular RNA of the skin cultures and a change from the production

of keratin to the production of cilia and mucus in the tracheal organ cultures. We have now investigated the biological activity of seven analogues of natural vitamin A ester (β -retinyl acetate) and vitamin A acid (β -retinoic acid), in line with the common practice of assaying physiological and pharmacological actions of vitamins, hormones and drugs using structural analogues of a parent compound. In the analogues we used, the 5,6-cyclohexenyl ring system of natural vitamin A is modified substantially. Although data obtained *in vivo* show that modification of the ring portion of the molecule can reduce growth-promoting activity³⁻⁵, we found that several vitamin A analogues with alterations in the ring, including a shift of the 5,6-double bond of the cyclohexene ring to the 4,5-position and replacement of the cyclohexene ring with substituted aromatic and cyclopentene rings, have substantial activity in the skin and tracheobronchial assays. Because of the sensitivity of the two assays it is now possible to assay vitamin A activity in the 10^{-8} – 10^{-10} M range (30–300 pg ml⁻¹). Moreover, there is excellent correlation between the results of the two assays in terms of the evaluation of biological activity of new analogues.

Vitamin A analogues (Fig. 1) were dissolved in dimethylsulphoxide (DMSO) and stored in a liquid nitrogen refrigerator. Growth and evaluation of epidermal cell cultures¹ and tracheal organ cultures² have been described in detail. The medium for epidermal cell culture was CMRL-1066 (ref. 6), containing 0.5% crystalline bovine albumin, 2 mM glutamine, penicillin (100 U ml⁻¹), and streptomycin (100 μ g ml⁻¹), for tracheal organ culture the medium was CMRL-1066, containing crystalline bovine insulin (1.0 μ g ml⁻¹), hydrocortisone hemisuccinate (0.1 μ g ml⁻¹), and glutamine, penicillin and streptomycin as above. The final DMSO concentration in epidermal cell cultures and tracheal organ cultures did not exceed 1.0% and 0.3%, respectively, all control cultures were treated with an equivalent amount of DMSO.

The effects of each vitamin A compound on RNA, DNA and protein content of epidermal cell cultures are shown in Table 1, their activity in reversing keratinised squamous metaplasia in tracheal organ culture is shown in Table 2. Data are reported for 230 cell cultures and 563 organ cultures. There is a good concordance in the results obtained in the two assay systems. Figures 2 and 3 show the type of dose-response profile that can be obtained in the epidermal cell culture assay. It is readily apparent that the 5,6-cyclohexenyl ring of naturally occurring vitamin A can be replaced with other structures without substantial loss of activity in the two assays. However, none of the synthetic analogues used had significantly greater activity than β -retinoic acid. Two analogues,

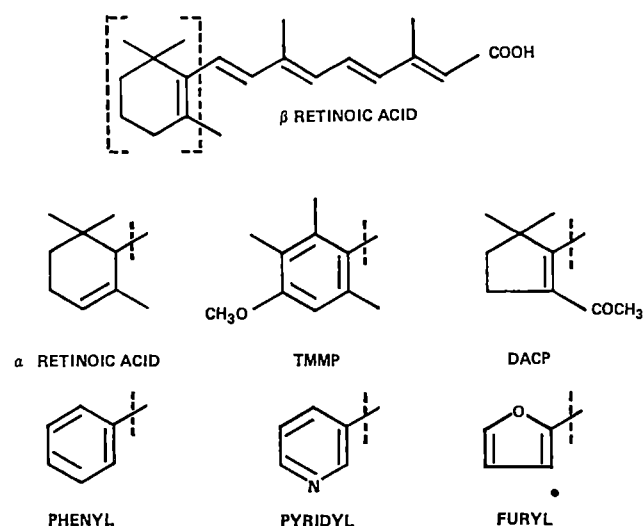


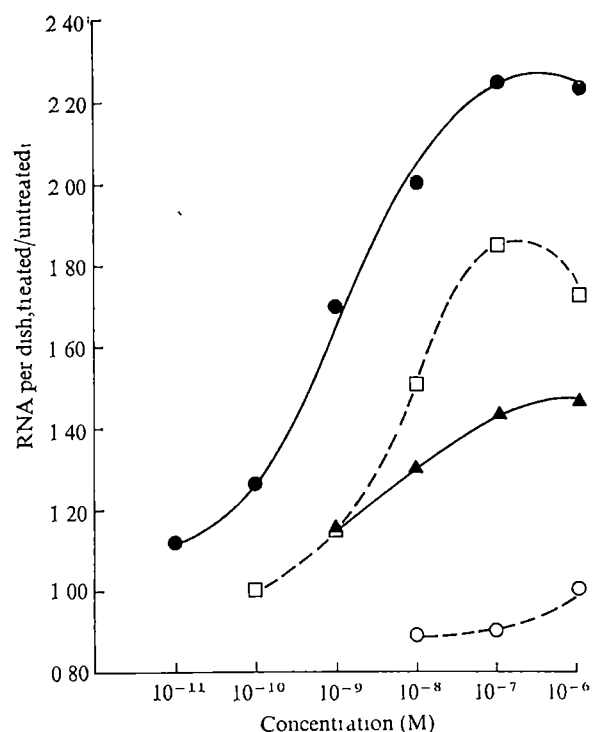
Fig. 1 Structures of β -retinoic acid and its analogues with modifications of the ring. In the analogues, the portion of the β -retinoic acid molecule shown in dotted brackets has been replaced with the structures shown.

Table 1 Percentage increase in RNA, DNA and protein in epidermal cell cultures treated with vitamin A analogues

Compound	concentration (M) (No. of cultures)	RNA	DNA	Protein
β-Retinoic acid				
10^{-6}	(12)	122	41	54
10^{-7}	(6)	125	46	54
10^{-8}	(12)	99	45	54
10^{-9}	(12)	70	28	44
10^{-10}	(9)	26	0	17
10^{-11}	(3)	12	-4	6
α-Retinoic acid				
10^{-6}	(6)	138	67	58
10^{-7}	(6)	96	44	26
10^{-8}	(6)	87	26	10
10^{-9}	(3)	42	0	3
10^{-10}	(3)	17	0	3
DACP analogue of retinoic acid				
10^{-6}	(6)	72	48	57
10^{-7}	(6)	84	54	69
10^{-8}	(6)	51	38	48
10^{-9}	(6)	16	21	31
10^{-10}	(3)	0	9	15
TMMP analogue of retinoic acid				
10^{-6}	(6)	148	55	33
10^{-7}	(6)	105	65	21
10^{-8}	(6)	79	51	17
10^{-9}	(3)	35	31	9
10^{-10}	(3)	26	31	9
10^{-11}	(3)	17	15	0
Phenyl analogue of retinoic acid				
10^{-6}	(9)	46	16	13
10^{-7}	(6)	44	23	20
10^{-8}	(6)	31	6	6
10^{-9}	(3)	17	—	—
Furyl analogue of retinoic acid				
10^{-6}	(6)	-1	1	14
10^{-7}	(2)	13	—	32
10^{-8}	(3)	0	—	7
Pyridyl analogue of retinoic acid				
10^{-6}	(9)	1	14	14
10^{-7}	(6)	-11	9	23
10^{-8}	(6)	-11	-6	23
β-Retinyl acetate				
10^{-6}	(6)	100	32	39
10^{-7}	(3)	116	46	44
10^{-8}	(6)	76	26	34
10^{-9}	(3)	36	5	14
10^{-10}	(3)	24	5	20
10^{-11}	(3)	12	0	9
α-Retinyl acetate				
10^{-6}	(3)	166	62	17
10^{-7}	(6)	64	21	3
10^{-8}	(6)	53	16	6
10^{-9}	(3)	12	0	3

Epidermal cells were grown for 3 d with the above vitamin A analogues. Values are expressed as the percentage increase in RNA, DNA or protein in the cultures, compared with control cultures which received no vitamin A. The mean values for such control cultures, after 3 d of growth, were as follows: total RNA per dish, 5.8 μ g, total DNA per dish, 9.3 μ g, and total protein per dish, 196 μ g. At the start of the experiment (zero-time controls), these mean values were: RNA, 5.8 μ g per dish, DNA, 7.2 μ g per dish, protein, 82.3 μ g per dish.

the furyl and pyridyl derivatives of retinoic acid, seemed to be almost devoid of activity, while the phenyl analogue had only minimal activity. There is no obligatory requirement for a 5,6-double bond in the cyclohexene ring, since both α -retinoic acid and α -retinyl acetate had marked activity in both assay systems. Similarly, both the dimethylacetylcyclopentenyl

**Fig 2** Dose-response curves, showing effects of both active and inactive vitamin A analogues on RNA content of mouse epidermal cell cultures. Structures of analogues are shown in Fig 1. ●, β -Retinoic acid, □, DACP, ▲, phenyl, ○, pyridyl.

(DACP) and trimethyl-methoxyphenyl (TMMP) analogues, in which the ring system has been even more substantially altered, also had marked activity in both assay systems. It is interesting that the DACP analogue of retinoic acid has been reported to block the hyperplastic and metaplastic effects of 3-methylcholanthrene on prostatic epithelium⁷, while the ethyl ester of the TMMP analogue of retinoic acid prevents skin carcinomas and papillomas in mice after exposure to polycyclic hydrocarbons⁸.

The results shown in Table 1 and Fig 2 suggest that the action of vitamin A in the differentiation of epithelial tissues involves an effect on RNA metabolism, we have reported⁹ an abnormal electrophoretic pattern of the high molecular weight RNA molecules synthesised in tracheal epithelium of vitamin A-deficient hamsters. Effects of vitamin A in stimulating nucleoside incorporation into RNA in other tissues have also been reported^{10,12}, but in each case the molecular site of action is unclear.

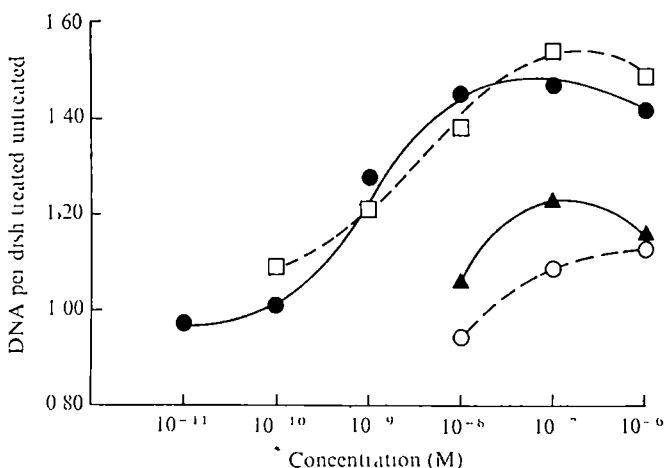
**Fig 3** Dose-response curves, showing effects of both active and inactive vitamin A analogues on DNA content of mouse epidermal cell cultures. Structures of analogues are shown in Fig 1. Symbols as in Fig 2.

Table 2 Reversal of keratinised squamous metaplastic lesions of vitamin A deficiency in tracheal organ cultures treated with vitamin A analogues

Treatment of cultures (No of cultures)	% Cultures with respective amounts of squamous metaplasia					% Cultures with keratin and keratohyaline granules
	None	Minimal	Mild	Marked	Severe	
No vitamin A, collected days 4-6 (87)	7	13	41	33	6	70
No vitamin A, collected days 11-13 (86)	2	4	33	44	17	91
β-Retinoic acid						
10 ⁻⁶ M (46)	48	41	11	0	0	0
10 ⁻⁷ M (22)	59	18	18	5	0	0
10 ⁻⁸ M (19)	21	16	42	11	11	0
10 ⁻⁹ M (11)	0	18	27	36	18	0
10 ⁻¹⁰ M (12)	0	8	42	33	17	75
α-Retinoic acid						
10 ⁻⁶ M (10)	60	30	10	0	0	0
10 ⁻⁷ M (15)	47	13	20	13	7	0
10 ⁻⁸ M (14)	7	7	50	36	0	0
DACP analogue of retinoic acid						
10 ⁻⁶ M (14)	57	0	36	7	0	0
10 ⁻⁷ M (19)	21	26	32	21	0	0
10 ⁻⁸ M (9)	11	11	44	33	0	0
TMMP analogue of retinoic acid						
10 ⁻⁶ M (18)	39	44	17	0	0	0
10 ⁻⁷ M (28)	29	7	46	18	0	0
10 ⁻⁸ M (14)	14	7	71	7	0	14
Phenyl analogue of retinoic acid						
10 ⁻⁶ M (14)	0	21	50	14	14	50
10 ⁻⁷ M (15)	7	13	33	27	20	67
Furyl analogue of retinoic acid						
10 ⁻⁶ M (13)	0	8	46	38	8	77
10 ⁻⁷ M (14)	0	14	43	29	14	79
Pyridyl analogue of retinoic acid						
10 ⁻⁶ M (13)	0	23	61	15	0	31
10 ⁻⁷ M (9)	0	11	33	44	11	89
β-Retinyl acetate						
10 ⁻⁶ M (13)	61	8	23	8	0	0
10 ⁻⁷ M (13)	15	31	31	23	0	8
10 ⁻⁸ M (11)	9	9	64	18	0	0
α-Retinyl acetate						
10 ⁻⁷ M (14)	21	14	36	29	0	0
10 ⁻⁸ M (10)	0	0	10	80	10	20

All tracheas were cultured for the first 4-6 d in medium without vitamin A. At this time, some tracheas were collected, while the rest were cultured for a further week in medium containing either no vitamin A or added vitamin A or vitamin A analogue. These tracheas were collected on the days 11-13 of culture. Cultures were graded as to the percentage of their total epithelium showing squamous metaplasia on eight cross sections from the middle of each trachea. If more than 40% of the total epithelial length was squamous, it was graded as having severe squamous metaplasia, between 10-40% was graded as marked, between 2-10% was graded as mild, and less than 2% was graded as minimal.

Obviously the *in vitro* systems for assay of vitamin A analogues do not necessarily give an evaluation of activity identical to that obtained with earlier tests of growth promotion in the whole animal. It has long been known that there may be a dissociation between the ability of a vitamin A analogue to support growth in the whole animal and to maintain specific epithelial structures, as in the case of β -retinoic acid and testicular epithelium^{13,14}. More recently, it has been shown¹⁵ that the DACP analogue of retinoic acid will not support growth in the rat, and yet we have found that this compound has definite vitamin A activity in both the epidermal and tracheal culture assays. Thus it seems that as new, more specific *in vitro* assay systems become available, the generic concept of vitamin A activity is becoming obsolete. It is increasingly essential to define the vitamin A activity of a specific chemical structure in terms of a specific biological or biochemical function, much in the same way as this was necessary some time ago for the understanding of structure-function relationships of adrenocortical steroids. The striking concordance of measured activity of vitamin A analogues in our two culture systems suggests that both systems measure a similar fundamental biological or biochemical property of related molecules.

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Thyroid hormone conformation from NMR studies of triiodothyropropionic acid

We have measured NMR spectra of triiodothyropropionic acid, a thyro-active analogue of the thyroid hormone triiodothyronine, to obtain information about the effects of differing solvent environments on hormone conformation

3,5,3'-Triiodo-L-thyronine (T_3) is the most potent naturally occurring thyroid hormone, and its conformational characteristics have been much studied. The steric effects of the iodine atoms favour a conformation with the iodinated phenyl rings lying in approximately mutually-perpendicular planes, and the 120° angle at the ether oxygen produces conformational distinction between the chemically identical 3' and 5' positions on the β ring. Thus the 3'-iodine of T_3 may be positioned either proximal or distal to the α ring (Fig 1). Although rotation of the β ring is possible, Jorgensen *et al*^{1,2} concluded from biological testing of conformationally fixed analogues of T_3 that the 3'-iodine distal arrangement is the important one at the hormonal receptors

An X-ray crystallographic determination of the three-dimensional structure of hydrated T_3 hydrochloride³ showed it to have the opposite conformation, that is the 3'-iodine is proximal to the α ring. Structure determinations of thyromimetic T_3 analogues, 3,5,3'-triiodothyropropionic acid ethyl ester⁴ and hydrated 3'-isopropyl-3,5'-diiodo-L-thyronine hydrochloride⁵ were performed and revealed both of these molecules also to have crystallised with conformations in which the 3'-substituent is situated in the proximal position. Subsequently, conformations with the β ring oriented with the 3'-iodine in the distal position were discovered in the crystal structure determinations of T_3 (ref 6), 3,5,3'-triiodothyroacetic acid-N-diethanolamine⁷ and T_3 methyl ester⁷

These results raise questions regarding the relative stabilities of the proximal and distal conformations, both in solid state and in solution, and the conditions under which each is favoured, and why. The crystals in which the 3'-substituent proximal positioning occurs were all obtained from acidic (HCl) solutions while the distal-conformation crystals were from alcohol with excesses of organic reagents often present. It seems therefore that the crystallisation medium plays a crucial role in promoting crystallisation of only one conformation or the other. Since crystallisation must be preceded by a local ordering in solution, it seemed likely to us that differences in the equilibrium populations of the proximal and distal conformers would exist in the different crystallisation solutions, and that these differences might be detectable using nuclear magnetic resonance (NMR) spectroscopy

We chose 3,5,3'-triiodothyropropionic acid (T_3P) as a representative T_3 structural analogue for the NMR spectral study in different solutions. The solutions were chosen to duplicate as closely as possible the conditions under which proximal and distal conformations of thyromimetics were crystallised, and consisted of (i) T_3P (~ 0.08 M) in a 2:1 mixture of ethanol-1N HCl, the medium from which proximal

triiodothyropropionate crystallised⁴, and (ii) T_3P (~ 0.06 M), diethanolamine (~ 0.06 M), and urea (~ 0.6 M) in methanol, the relative conditions under which distal triiodothyroacetic acid-N-diethanolamine was obtained⁷. The molar concentration of T_3P used was the maximum that could be obtained under the given conditions. The spectra were recorded at room temperature on a 220 MHz spectrometer, with tetramethylsilane as an internal reference

Figure 2 shows the NMR spectra of the aromatic-ring protons of T_3P in the two solutions. Peak assignments are labelled on the figure, and the chemical shifts are given in Table 1. The spectrum of T_3P in methanol-urea-diethanolamine is indicative of either only one species being present, or, possibly, a time-averaged distribution of species whose inter-conversion time (by β ring rotation) is very fast. The spectrum of T_3P in acidic ethanol, however, shows doubling of all the proton peaks, and is consistent with an interpretation of two distinct species being present, with a major:minor species ratio of about 3:1. The peaks due to the 2'-hydrogen of the major

Table 1 Chemical shifts p.p.m. of aromatic ring hydrogen atoms in T_3P NMR spectra

Atoms	CH ₃ OH-diethanolamine	CH ₃ CH ₂ OH-HCl	
	urea	Major peaks	Minor peaks
H _{6'}	6.57	6.560	6.544
H _{5'}	6.76	6.816	6.810
H _{2'}	7.01	7.000	7.028
H _{2,6}	7.81	7.760	7.775

species are displaced upfield from those of the minor component while the 5'- and 6'-proton peaks of the major species are downfield from the corresponding peaks of the minor entity, this is what would be expected if the 2'-proton of the major species were closer to the α ring and the 5'- and 6'-protons farther from the α ring than the corresponding atoms in the minor species. Thus the major entity is indicated to be the 3'-iodine proximal conformation, and the minor species the distal one. (Even the relative amounts of the displacements of the corresponding peaks of the two species agree with the relative distances of the protons from the α ring in each case.)

We also recorded a higher temperature NMR spectrum of T_3P in ethanol-HCl to observe the stabilities of the species present. No significant coalescence of the peaks occurred at approximately 60° C.

The above interpretation is consistent with and substantially clarifies the crystallographic results. In acidic (HCl) solutions the 3'-substituent proximal conformation predominates, and that is the conformation which crystallises from such media, in the other medium, from which the distal conformation crystals were obtained, only a single-species spectrum is obtained. If this represents a time-averaged distribution presumably the distal conformation is favoured (or perhaps just less soluble), but if so, the energy difference is too small for both species to be isolated by NMR at room temperature. The NMR results also pose some problems: for example (i) why does acidification of the medium cause the stabilisation of the 3'-iodine proximal arrangement, and (ii) why is the energy barrier for interconversion of the species in acidic media so high—the non-coalescence

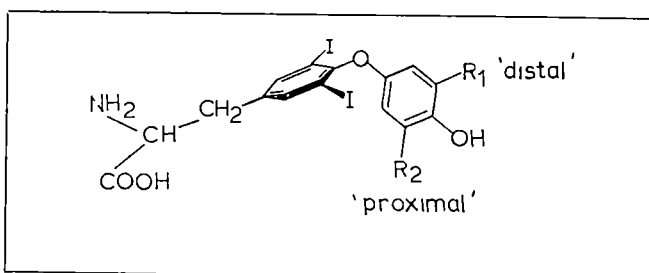


Fig 1 Proximal and distal conformations of asymmetric β ring thyromimetics

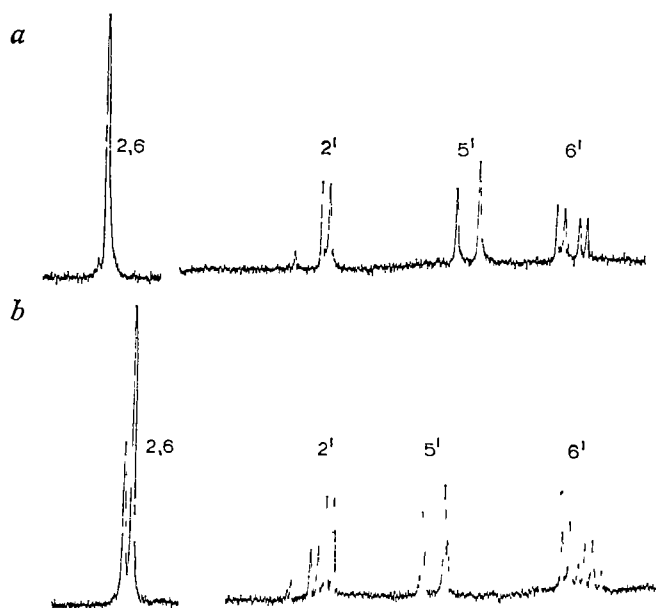


Fig. 2 NMR spectra of the aromatic ring hydrogen atoms of triiodothyropropionic acid in methanol-urea-diethanolamine (a) and ethanol-HCl (b). The field increases to the right.

of the splittings at 60° C implies a free energy of activation of at least 20 kcalorie mol⁻¹ (calculated from the 5'-hydrogen peaks separation). These questions and others pertaining to the effects of differing milieus on conformational stability are being investigated in our laboratories by systematic NMR studies at controlled pH and temperatures.

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Increased axoplasmic flow associated with pargyline under conditions which induce a myopathy

The suggestion that an abnormality of the circulation might be important in the pathogenesis of Duchenne muscular dystrophy¹⁻³ has been supported by the abnormal intrafibrillar fluorescence of muscle biopsies⁴, which is presumed to be due to the presence of catecholamines⁵. Accumulation of cate-

cholamines, accompanied by the appearance of a myopathy, can be induced by the monoamine oxidase inhibitor pargyline⁶, while this myopathy can be prevented by sectioning of the sciatic nerve. This suggests that the induced myopathy is dependent on an intact nerve supply to the muscle and that catecholamines are involved in the process. Studying fast axoplasmic flow in rats treated with pargyline, we have obtained evidence of altered nerve function.

Male Sprague-Dawley rats (150-170 g) were given daily intraperitoneal injections of pargyline. The drug solution was prepared each day by dissolving 20 mg ml⁻¹ of normal saline and injecting 75 mg kg⁻¹ body weight⁵. Fast axoplasmic flow was studied in rats anaesthetised with pentobarbital (50 mg kg⁻¹) by injecting ³H-L-leucine (44.2 Ci mmol⁻¹, New England Nuclear Corp.) into the right ventral horn of the exposed spinal cord at spinal levels L-5 and L-6 as described by Lasek⁶. The animals were killed after 0.5-2.5 h, and the sciatic nerve and the L-5 and L-6 spinal roots were dissected out intact, cleaned of connective tissue and frozen immediately on a steel ruler using a Cryoquick spray. The frozen nerve was cut into 3-mm segments which were dissolved in 1 ml of Soluene 350 (Packard), 10 ml PCS (Amersham/Searle) was added and the samples were counted.

Examples of the distribution of radioactivity expressed as d.p.m. per 3-mm nerve segment as a function of the distance along the sciatic nerve are shown in Fig. 1. In the rat given saline as a control, a labelled front migrated 40 mm in 2.5 h, while 24 h after a single dose of pargyline the radioactive front extended to 35 mm within the first 1.5 h. Furthermore, as the number of doses of pargyline increased, the rate of transport of ³H increased to 52 mm h⁻¹ for rats exposed to pargyline for 3 d and 46 mm 0.5 h⁻¹ for those exposed for 7 d. For both groups the flow of material down the nerve appears to have separated into multiple fronts. This might be due to drug-induced alterations in the rate of migration of different populations of ³H-containing molecules, that is proteins and glycoproteins, both being transported by fast axoplasmic flow⁷. Slow axoplasmic

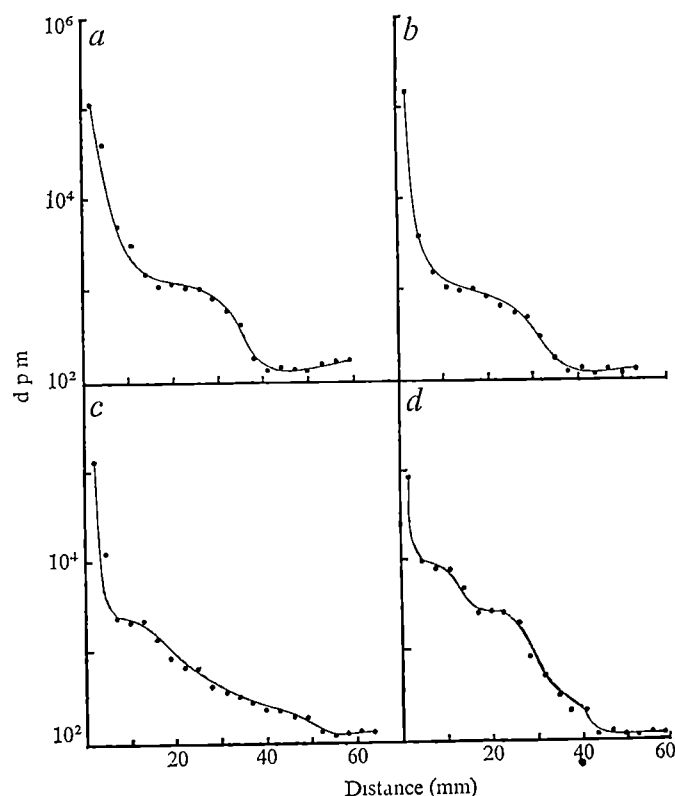


Fig. 1 Distribution of protein-bound radioactivity along the sciatic nerve of NaCl control (a) and 1 (b), 3 (c) and 7 d (d) pargyline-treated rats. Animals were killed at 2.5 h (a), 1.5 h (b), 10 h (c) and 0.5 h (d) respectively after labelling the spinal cord with ³H-leucine.

Table 1 Pargyline-induced increase in fast axoplasmic transport of ^3H -labelled material in the rat sciatic nerve

Pargyline 75 mg kg ⁻¹			Pargyline 25 mg kg ⁻¹		NaCl control
1 d	3 d	7 d	3 d	7 d	7 d
552±9 n = 4	1,262±117 n = 7	1,996±270 n = 8	877±45 n = 3	1,428±35 n = 4	390±13 n = 5

Figures are mm 24 h⁻¹±s.d

flow⁸ does not appear to play a role in the development of this myopathy because the rate of slow flow is 1–2 mm d⁻¹ and necrosis is evident already after the first day of pargyline administration⁵

The rate of migration of ^3H down the nerve was related to the number of days the rats were exposed to pargyline increasing from 1 to 7 d, and was affected by the dose received each day (Table 1). The response elicited by pargyline could be duplicated by another monoamine oxidase inhibitor phenelzine (40 mg kg⁻¹) suggesting that an increase in monoamine levels was involved in the increase in the rate of axoplasmic flow. This was supported by our observation that pretreatment with α -methyl paratyrosine (200 mg kg⁻¹) decreases by 30% the increase in rate of fast axoplasmic flow induced by pargyline given for 3 d.

An abnormally large amount of catecholamines excreted in the urine⁹ and present in muscle⁴ of genetically dystrophic patients has been documented. In addition a model has been presented³ suggesting that a vascular abnormality with an increased sensitivity to the level of catecholamines is responsible for the development of an experimental myopathy similar to that found in Duchenne dystrophy. Monoamines are important inhibitory mediators in the central nervous system^{10,11}, and in patients with Duchenne dystrophy fewer than the usual number of functional motor units have been reported¹². We suggest that a rise in monoamine levels induced by monoamine oxidase inhibitors activates the inhibitory response in the central nervous system leading to depression of motor neurones in the spinal cord and therefore a decrease in the level of muscle activity. This could lead to an increased demand by the muscle for trophic factors which would in turn increase the rate of axoplasmic flow. A similar mechanism could explain some of the findings reported for Duchenne muscular dystrophy.

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New pathway for metabolism of dopa

L-DOPA (3, 4-dihydroxyphenylalanine) has, since 1967, been used in large doses clinically to lessen the degree of akinesia, rigidity and tremor in Parkinson's disease¹. The impetus for dopa therapy came from the discovery of abnormally small concentrations of dopamine in the basal ganglia of patients with Parkinsonism². Since catecholamines cannot cross the blood-brain barrier, dopa, the immediate metabolic precursor of dopamine, became the logical candidate for clinical trials. Administration of dopa increases dopamine in the basal ganglia³, and this is believed to be the primary mode of action of the drug. Although partially correct, this cannot account for the so-called on-off effect⁴—the increase in therapeutic benefits derived from prolonged use of dopa⁵—and side effects such as nausea, vomiting and personality disturbances. The latter are minimised by simultaneous administration of decarboxylase inhibitors which limit peripheral decarboxylation and allow smaller doses of dopa to be effective. Nonetheless it seems that the action of dopa is complex, with several mechanisms operative. It is therefore important to elucidate all possible metabolic fates of dopa and consider their relationship to the various effects of dopa. We report here *in vitro* evidence for a minor pathway of dopa metabolism resulting in the formation of 3,4-dihydroxyphenylacetaldehyde (DHPA) with concomitant inactivation of dopa decarboxylase.

α -Methyldopa, a poor substrate of dopa decarboxylase, inactivates this pyridoxal 5'-phosphate (PLP)-dependent enzyme⁷ as a result of abortive transamination⁸, a side reaction producing apoenzyme, pyridoxamine 5'-phosphate (PMP) and the aldehyde or ketone (in this case, 3,4-dihydroxyphenylacetone) derived from decarboxylation and transamination of the substrate. First observed in the decarboxylation of α -methylglutamate by glutamate decarboxylase⁹, abortive transamination was found to occur to a lesser degree with glutamate itself¹⁰. Mechanistically, transamination follows decarboxylation if protonation of the enzyme-bound Schiff's base occurs at C₄ instead of C _{α} (Fig. 1).

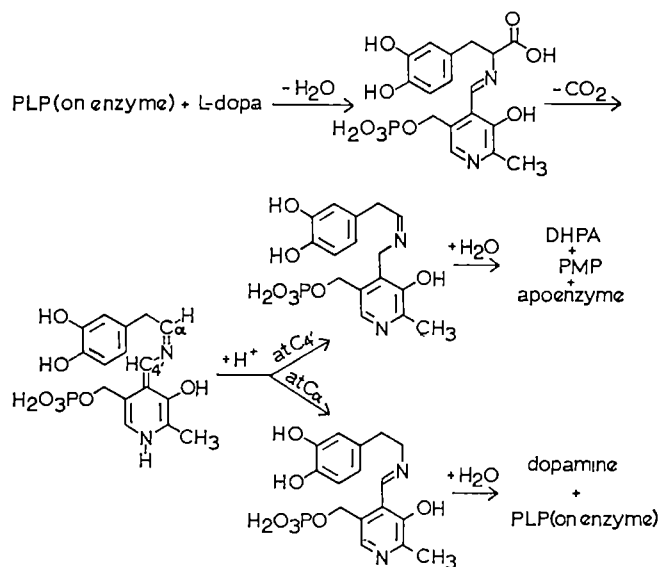


Fig. 1 Protonation of the enzyme-bound Schiff's base at C₄ and C _{α}

Hydrolysis of the Schiff's base then yields an aldehyde or ketone and PMP, which dissociates, leaving apoenzyme. Abortive transamination may be a general reaction of PLP-dependent decarboxylases with all substrates. Because several grams of dopa per day must be metabolised by patients on dopa therapy, even a small fraction of the turnover resulting in production of DHPA and inactivation of dopa decarboxylase could be

Table 1 Ratio of radioactivity added to that isolated

Experiment	c p m added as ^3H -dopa	c p m isolated as 2,4-dinitrophenylhydrazone	% Transamination
1	4.7×10^7	4.1×10^4	0.009
2	4.9×10^7	6.7×10^4	0.014
3	4.6×10^7	6.2×10^4	0.013
4	4.9×10^7	6.0×10^4	0.012
			Average 0.012

Dopa decarboxylase was purified partially from hog kidney homogenate to a specific activity of 1,000 U mg^{-1} (1 U = 1 nmol CO_2 produced per min) using a procedure combining aspects of published protocols^{11,12}. In a typical experiment to detect DHPA, the enzyme was incubated in 5.0 ml 0.1 M phosphate buffer, pH 7.0, containing 10^{-4} M PLP, 2 mg ^3H -dopa, synthesised¹³ by acid-catalysed exchange of aromatic protons with T_2O , in 0.4 ml buffer was added, and the reaction proceeded for 5 min. Sufficient enzyme was present to decarboxylate >90% of the substrate in this time. After addition of a small amount of unlabelled DHPA¹⁴ as a carrier, the solution was extracted with two 5-ml portions of ethyl acetate. The combined extracts were dried over Na_2SO_4 and were concentrated to approximately 50 μl with a rotary evaporator. This solution was applied to the corner of a 10×10 cm silica gel thin layer chromatography plate and was chromatographed with ethyl acetate. An ethanolic solution of 2,4-dinitrophenylhydrazine was sprayed on the spot corresponding to DHPA ($R_F = 0.54$), and a second chromatography, using ether as a solvent, was done at right angles to the original direction of elution. The orange spot ($R_F = 0.46$) corresponding to the 2,4-dinitrophenylhydrazone of DHPA was scraped from the plate and counted.

physiologically significant. We therefore investigated whether dopa decarboxylase catalyses this abortive transamination of dopa.

The detection of DHPA as a by-product of the enzymatic decarboxylation of dopa is described in the legend of Table 1. We used ^3H -dopa of high specific activity (0.1 mCi mg^{-1}) and isolated the somewhat unstable aldehyde as its 2,4-dinitrophenylhydrazone, which contained significant amounts of label. Table 1 compares the c p m found in the derivative with the c p m added initially as ^3H -dopa. The ratio indicates that approximately one in every 10^4 decarboxylations also results in transamination. Because of facile oxidation of the aldehyde and the semi-quantitative nature of the isolation of the phenylhydrazone, this represents a minimum value. If less enzyme was used, the radioactivity isolated was proportional to the amount of enzyme. When this experiment was done without enzyme or with heat-inactivated enzyme, less than 10% as much radioactivity was found in the phenylhydrazone. With a large excess of enzyme there was no additional radioactivity, eliminating the possibility that a trace amount of monoamine oxidase was responsible for production of DHPA.

In the absence of free PLP, dopa decarboxylase is inactivated by the abortive transamination. Enzyme freed of unbound PLP by gel filtration was incubated with 2×10^{-3} M dopa for various times. Remaining decarboxylase activity was determined by addition of 1.0 μmol of $1\text{-}^{14}\text{C}$ -dopa and collection of $^{14}\text{CO}_2$ evolved during the next 5 min. Inactivation by dopa occurred with $t_{1/2}$ of 6.6 min, comparable with the 2 min found for α -methyl-dopa⁸.

Addition of PLP to these partially inactivated solutions of enzyme showed that 30–50% reactivation was possible. The remainder of the original activity was lost, presumably because of the instability of the apoenzyme. There was no loss of activity when the enzyme was incubated at 37°C for 30 min in the absence of both free PLP and substrate.

The percentage of catalytic events leading to transamination can be calculated independently from the rate of inactivation. Assuming a maximum specific activity¹¹ of 9,000 U mg^{-1} , a molecular weight¹¹ of 112,000 and a $t_{1/2}$ for inactivation of 6.6 min, we calculate 0.01% for transamination against normal reaction—in agreement with the directly measured value.

The abortive transamination of dopa by dopa decarboxylase has been characterised by the demonstration of DHPA, inactivation of the enzyme in the absence of free PLP at a rate

commensurate with the amount of DHPA formed and the ability of PLP to reactivate the enzyme at least partially. The fractional turnover leading to transamination together with decarboxylation is small, but measurable.

Several questions can be asked concerning the importance *in vivo* of the abortive transamination of dopa. First, does DHPA arising from this pathway have physiologically significant effects which differ from those of DHPA formed by the oxidation of dopamine? Second, does the inactivation of dopa decarboxylase by this mechanism cause inhibition of dopa metabolism either peripherally or in the brain? Finally, if such inhibition indeed occurs, will the metabolism of other substrates of dopa decarboxylase be affected? The answers to these questions, when available, may aid in an understanding of dopa metabolism and Parkinsonism.

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Histamine release by gastric stimulants

It is a matter of debate whether histamine in the gastric mucosa acts as a local chemical mediator for other gastric stimulants^{1–5}, and the isolated bullfrog mucosa provides a convenient model for investigation of the question. The preparation responds to gastric stimulants such as pentagastrin, acetylcholine and histamine, the secretory rate can be monitored easily and there are no complicating factors such as changes in blood flow. Nevertheless, conflicting reports have appeared. Davidson *et al.*⁶ found no changes in histamine content after addition of a gastrin-like pentapeptide, while Kasbekar *et al.*⁷ showed that pentagastrin and acetylcholine increased the efflux of labelled histamine. I have now used a more direct approach and found that histamine is released in response to pentagastrin and acetylcholine for which it seems to be a common mediator.

Isolated mucosa of *Rana catesbeiana* was mounted as a sheet between two sides of a leucite chamber. The serosal side had a capacity of 2.5 ml whereas the mucosal side could hold up to 4.5 ml. The normal serosal solution consisted of (mM) 89.4 NaCl, 3.0 KCl, 0.8 MgCl_2 , 18.0 NaHCO_3 , 1.8 CaCl_2 and 11.0 glucose. In the mucosal solution, glucose was omitted and NaHCO_3 was replaced by NaCl. Serosal solution was bubbled continuously with 95% O_2 /5% CO_2 and the mucosal solution

with 100% O₂. The area of the stomach exposed to the bathing solutions was 2.85 cm². The rate of secretion was monitored by a pH stat set at 7.7 as described earlier⁸. Freshly mounted stomachs secreted acid at rates varying from 6–10 $\mu\text{eq h}^{-1}$. With periodic changes of solutions, the rates of acid secretion declined during 8–12 h. Stomachs that secreted acid at rates of 2 $\mu\text{eq h}^{-1}$ or less, defined as 'resting', responded to exogenous stimulants added to the serosal solution⁹.

To estimate histamine in the serosal solution, the contents of the entire chamber were collected at various times. The starting or initial value was obtained on a sample collected in the first hour after dissection. After the stomachs had come to rest, a 30-min sample was collected ('control'). The serosal solution was then replaced with one containing one of the following stimulants: pentagastrin (5×10^{-6} M), acetylcholine (10^{-3} M) or dibutyl cyclic AMP (5 mM). These concentrations were used to obtain an optimal increase in acid secretion. Samples were collected at 15 or 30-min intervals. After each collection, the serosal side was refilled with the solution containing the same stimulant. Aliquots (1 ml) of the serosal solution were used to estimate histamine fluorometrically with an Aminco fluorophotometer¹⁰. The method depends on the production of a fluorophore by condensation of histamine with *o*-phthalaldehyde (*o*-PT). The fluorescent and activation spectra of the fluorophore obtained with the serosal solution and with standard histamine were indistinguishable. Appropriate blanks containing the stimulants were run concurrently, these usually gave negligible results except with solutions containing dibutyl cyclic AMP. The values of histamine obtained by these assays are expressed in terms of ng h⁻¹. This corrects for the different times of collection and also allows better correspondence with rates of acid secretion expressed as $\mu\text{eq h}^{-1}$. Freshly mounted stomachs secreted acid at a rate of $5.8 \pm 0.5 \mu\text{eq h}^{-1}$ ($n = 11$). The histamine liberated during that period was $498 \pm 78.8 \text{ ng h}^{-1}$. Resting stomachs secreted acid more slowly ($1.7 \pm 0.12 \mu\text{eq h}^{-1}$) and also had lower rates of histamine liberation ($135.5 \pm 12.6 \text{ ng h}^{-1}$).

Addition of pentagastrin or acetylcholine to the serosal solution led to a marked increase in the liberation of histamine and acid (Fig. 1), which reached a maximum and then declined. The release of histamine reached a peak in the first 15 min and then declined, and by the end of an hour it was near control values. In contrast, the rate of acid secretion was maximal after 25–30 min and even at the end of the hour it was significantly higher than the resting value. This suggests that the release of histamine

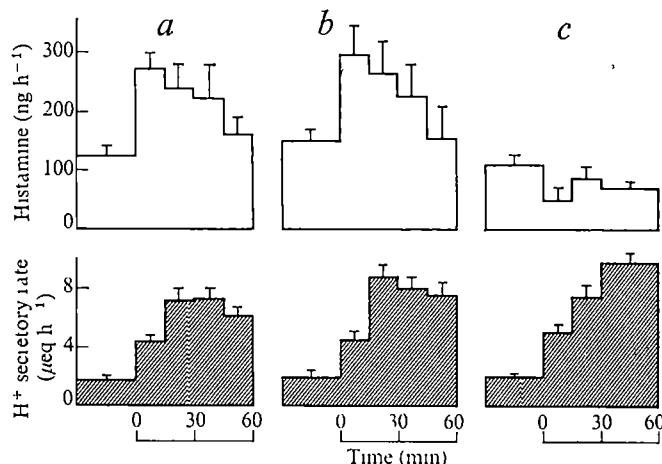


Fig. 1 Effects of adding (a) pentagastrin (5×10^{-6} M), (b) acetylcholine (10^{-3} M) or (c) dibutyl cyclic AMP (5 mM) to a resting frog stomach are shown. The drugs were added at time zero in each case. Samples were collected at 15 min intervals in the case of pentagastrin or acetylcholine. After each collection, the serosal side was refilled with the solution containing the same stimulant. The values shown are the means \pm s.e.m. for five experiments. Where dibutyl cyclic AMP was used (four experiments), two samples were collected at 15-min intervals and the third after 30 min.

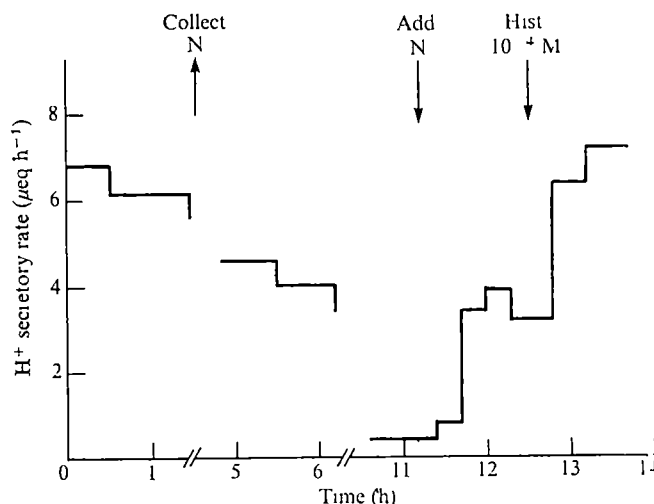


Fig. 2 Increase in acid secretion on readdition of serosal (nutrient) solution to a resting stomach. A 90-min sample was collected early in the day (collect N) when the stomach was secreting spontaneously at a high rate. The sample was stored in the cold and added back after the stomach had come to rest (add N). Sixty minutes later, 10^{-4} M histamine was added to the serosal solution.

preceded the onset of acid secretion. Dibutyl cyclic AMP increased the rate of acid secretion but produced no significant increase in the release of histamine.

Figure 2 shows the results of an experiment in which a stomach was mounted as before, but after 30 min the serosal chamber was drained and 2.5 ml of fresh solution was added. After 90 min, the entire contents of that chamber were collected, labelled (N), and stored in the cold. After the stomach had come to rest, the serosal chamber was drained and refilled with the sample collected earlier, and allowed to warm to room temperature. As Fig. 2 shows, addition of the sample led to a marked increase in secretion of acid, which increased from $1.3 \pm 0.3 \mu\text{eq h}^{-1}$ to $3.8 \pm 0.7 \mu\text{eq h}^{-1}$ in four experiments. The stomachs also responded to exogenous histamine, 10^{-4} M. These experiments (a variation of the Loewi experiment on Vagustoff) show that the material that leaks out from the stomach into the serosal solution can stimulate acid secretion. The results of the first experiment suggest that the material is histamine.

The following conclusions can be drawn from this study. Histamine leaks from the frog gastric mucosa into the serosal solution in measurable amounts. The spontaneous secretion of the frog stomach mounted in chambers is due to histamine liberated either during pithing or dissection. The gradual decline in acid secretion presumably represents slow diffusion or metabolic degradation of the liberated histamine. The stores of histamine are not exhausted, however, and exposure to pentagastrin or acetylcholine leads to a transient release of histamine. The histamine that is released stimulates acid secretion, presumably by activating adenylcyclase¹¹. Dibutyl cyclic AMP acting at a stage beyond that of histamine release activates acid secretion directly. Thus histamine appears to be the common mediator for acetylcholine and pentagastrin in the frog stomach.

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Dissociation of Factor VIII-related antigen into subunits

FACTOR VIII-related antigen is present in normal human blood plasma and with conventional separation procedures it is detected in the same fractions as Factor VIII, the active blood clotting factor. Its structure and absolute concentration in plasma are unknown but it is believed to be a protein of high molecular weight. Patients with von Willebrand's disease have antigen levels and Factor VIII levels reduced in approximately the same proportions while haemophiliacs have reduced amounts of Factor VIII but normal concentrations of antigen. A differential diagnosis between these two bleeding diatheses can therefore be made by measuring antigen level. It has been suggested that the reduced amounts of Factor VIII-related antigen in von Willebrand's patients may be responsible for some aspects of the bleeding encountered with this disease and also for some of the platelet abnormalities which are found.

It has already been shown that Factor VIII readily separates into subunits under the influence of concentrated saline solution^{1,2} or reducing agents^{3,4} and the antigen remains as a large intact molecule when the Factor VIII is dissociated. This illustrates a clear distinction between the two species and a method of separating them. Here we show that it is possible to dissociate Factor VIII-related antigen and that this dissociation may be detected using either immunoelectrophoresis techniques or the 'ristocetin aggregation test'.

Human Factor VIII used in these experiments was a concentrate made according to the method described by Newman *et al*⁵ for a material of intermediate potency. It was dissolved in 0.15 M saline to make a solution containing 10-20 units of Factor VIII activity ml⁻¹ (1 unit is defined as the amount present in 1 ml of average normal human plasma). When a sample of this starting material is subjected to gel filtration on Sepharose 4B the Factor VIII-related antigen appears in the first fraction, the so-called 'void volume'. Results of such an experiment are given in Fig 1 where antigen concentration is plotted against volume of eluate. The column dimensions were 260 mm × 15 mm and the chromatographic procedure was identical to that described previously⁴. Antigen is measured here as a percentage of the applied sample so that a total of all plotted values equals the overall recovery which in this case is 44%. Two methods of measurement were used, one was the immunoelectrophoretic method described by Zimmerman *et al*⁶ which is based on the Laurell technique⁷. The second method was the measurement of the effect on platelet aggregation by Ristocetin^{8,9}. It will be seen that both methods give the same results in terms of position and character of the eluted peak. Peak position is equivalent to 13.5 ml eluate which indicates a large molecular size probably greater than one million. Factor VIII itself gives a peak in the identical position⁴.

Factor VIII concentrate was reacted for 1 h at 37°C with 10⁻³ M dithiothreitol in 0.15 M saline. No buffers were

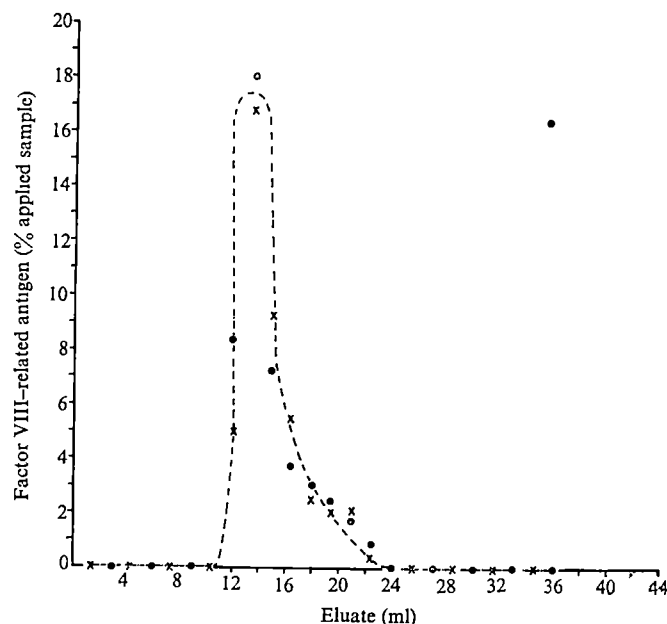


Fig. 1 Gel filtration of Factor VIII-related antigen before reaction. Antigen measured by immunoelectrophoresis (●) and ristocetin aggregate of platelets (×).

used but the solutions all had a pH between 6.5 and 7.0. After this incubation period the mixture was loaded on to a Sepharose 4B column, previously equilibrated with 10⁻³ M dithiothreitol in 0.15 M saline and the column was eluted with the same solution. In these conditions the elution pattern seen in Fig 2 was obtained. Now the antigen appears at a point equivalent to 26 ml of eluted volume indicating that the dithiothreitol treatment has dissociated it into subunits (total recovery = 40%). Comparison with previous work⁴ shows that this elution point is the same as that obtained for Factor VIII subunits and indicates that the molecular weight of the antigen subunit is identical to that of the Factor VIII subunit at least to within ± 25%. Calibration of the column using materials of known molecular weight showed that this elution peak position was equivalent to a molecular weight of 230,000. As with the intact molecule, results obtained using the two different methods of measurement were identical.

The level of dithiothreitol used to dissociate Factor VIII-related antigen in this work was 10⁻³ M as discussed earlier. At this concentration, Factor VIII clotting activity is destroyed, most of the destruction taking place in passage of the reaction mixture through the column (which is eluted with 10⁻³ M dithiothreitol). Factor VIII itself is dissociated at 1 × 10⁻⁴ to 3 × 10⁻⁴ M dithiothreitol⁴ for the samples in question and at this concentration the Factor VIII-related antigen remains intact as a large molecule. Thus there is a very clear difference between the antigen and Factor VIII even though it appears to be of degree. Unlike Factor VIII, it has not been possible to dissociate the antigen using high concentrations of sodium chloride but this is complicated by the fact that high saline concentrations precipitate proteins. Unlike Factor VIII it has not been possible to aggregate the antigen subunits using ε-aminocaproic acid.

There has been considerable discussion as to whether the two methods of measurement used in this work detect the same molecular species or not. As shown above these methods give identical results for the dissociated as well as for the undissociated antigen, so it must be concluded that they detect either the same or else very closely related species.

Dithiothreitol is a well known reducing agent, so it can be speculated that the subunits of Factor VIII-related anti-

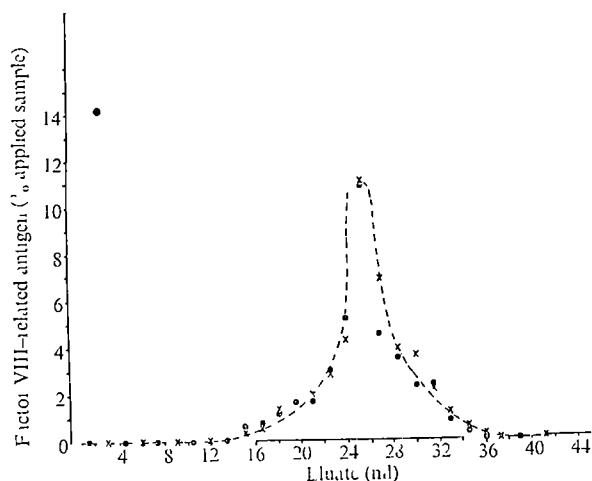


Fig 2 Gel filtration of Factor VIII-related antigen after reaction with 10^{-3} M dithiothreitol. Antigen measured by immunoelectrophoresis (●) and ristocetin aggregation of platelets (x)

gen are joined by disulphide linkages and that these are severed by reduction in the dissociation process. A possible alternative, however, is that reduction of certain parts of the molecule have made the subunits less capable of fitting together. This possibility cannot be disregarded at the present time as such an explanation might be more acceptable in the case of Factor VIII itself when reactions other than reduction will cause dissociation.

It is becoming clear that there is an intriguing analogy between Factor VIII-related antigen and Factor VIII itself. The similarity in the size of subunits and the probable similarities in the intact molecule might suggest that they belong to the same class of compound. It could also be speculated that they each have the same number of subunits per molecule and this would be contrary to the hypothesis put forward that a single Factor VIII subunit is carried by the intact antigen. In spite of these similarities, however, there must be a distinct difference in the strength of cohesive forces binding the subunits to account for the fact that more strenuous conditions are required to dissociate the antigen. Thus, Factor VIII-related antigen and Factor VIII itself are not identical. M A H acknowledges the provision of a grant by the Medical Research Council.

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Modulation excitation spectro-photometry of purple membrane of *Halobacterium halobium*

When *Halobacterium halobium* is grown at low oxygen concentrations in the light, it synthesises patches of membrane containing a purple pigment. If exposed to low salt concentrations, the cell membrane dissociates into fragments which differ in their protein and pigment composition and can be separated¹. The most conspicuous of these fragments is the so-called 'purple membrane'². The isolated purple membrane contains 25% lipid and 75% protein², only a single species of protein has been found. This protein, bacteriorhodopsin, is apparently similar to the animal visual pigment, it contains 1 mol of retinal per mol protein bound as a Schiff base to a lysine residue³. The protein forms a planar lattice in the membrane⁴ and shows a broad absorption maximum at 570 nm^{3,4}. Absorption of light converts the 570 nm species to a second species which absorbs maximally at 412 nm, and in the dark reconverts to the 570 nm complex within a few milliseconds. This is apparently accompanied by a conformational change in the protein, which cycles rapidly between the two conformations, releasing protons on one side of the membrane in the first transition and taking them up on the other in the second⁵. Thus, in the intact cells the bacteriorhodopsin seems to act as a light-driven proton pump^{6,7}.

The purple membrane has been the object of several spectrophotometric studies designed to elucidate the mechanism and kinetics of the photochemical reaction. These have usually been carried out by d.c. or flash methods and often at low temperatures or in solvents chosen to arrest the decay of the photo-transients^{5,8}. Using low-temperature spectroscopy, Lozier and Stoekenius have observed four spectrally distinct intermediates analogous to the rhodopsin, prelumirhodopsin, lumirhodopsin and metarhodopsin of invertebrates⁹. The two additional forms

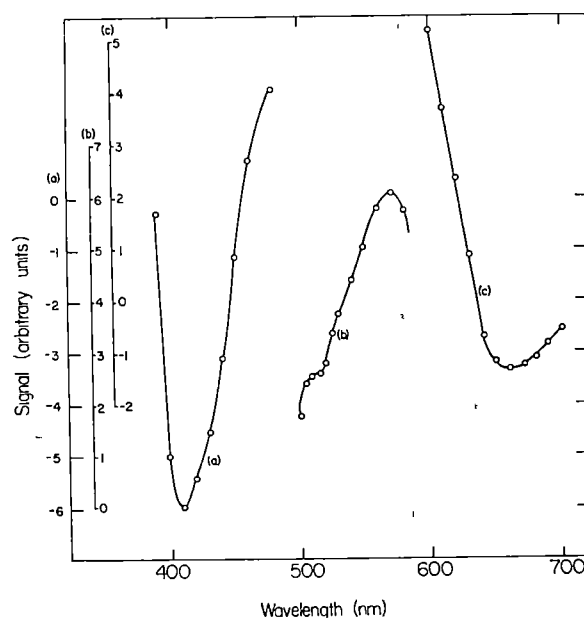


Fig 1 Absorption spectra of transients in purple membrane suspensions in water (room temperature). Modulation frequency 5 Hz. Scales are in arbitrary units, true ratios between the peaks are -11 ± 23 for 410 nm/570 nm/660 nm. Excitation was by a 250 W high-pressure mercury arc with emission extending across the ultraviolet and visible regions. Similar results were obtained by excitation at 365 nm, using a standard filter. Purple membrane suspensions were prepared from *H. halobium* R₁ as described by Danon and Stoekenius⁷, the concentration of purple complex was 0.03 mM according to A measurements based on millimolar absorption coefficient of $63 \text{ mM}^{-1} \text{ cm}^{-1}$ at 568 nm⁵. Assuming a 1:1 correspondence between the bands at 410 nm and 570 nm, their molar absorption coefficients should be in the ratio 11:23. This compares well with the ratio 11:21 for the 415 nm and 568 nm peaks according to the data of Oesterhelt and Hess⁵.

of bacteriorhodopsin were observed to have absorption bands centred at 610 nm and 550 nm, with a maximum in the difference spectrum at 510 nm. Resonance Raman spectroscopy has indicated that a Schiff-base linkage may be directly involved in the proton translocation¹⁰. Here low-temperature and solvent trapping techniques were used to study the 412 nm complex.

Modulation excitation spectrophotometry offers a convenient way of studying systems of this kind under more natural circumstances. Moreover, the light intensities used are much lower than in the case of flash photolysis. The method has been described in detail^{11,12} and briefly consists of illuminating the sample with a modulated light while at the same time observing the modulation on a constant monitoring beam, thus allowing the absorption of phototransients to be obtained. The difference of phase between the excitation modulation and that of the monitoring beam enables kinetics to be studied. Furthermore, by exciting through a polariser and analysing the monitoring beam, the polarisation of the transients may be determined.

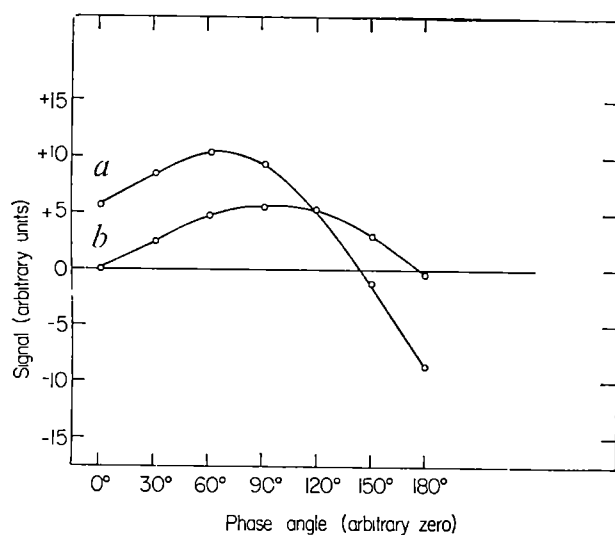


Fig 2 The two components of polarisation at 410 nm as a function of phase angle. Modulation frequency 108 Hz. *a*, Parallel polarisation, *b*, perpendicular polarisation.

The modulation excitation spectra of a suspension of purple membrane fragments in water at room temperature are shown in Fig 1, measured at 5 or 10 nm intervals. It can be seen that there are bands associated with the phototransients at 410 nm, 510 nm and 660 nm. In addition, the positive depletion band of the original pigments is observed at 570 nm. Although the 410 nm and 660 nm absorption bands are clearly resolved, that at 510 nm only appears as a shoulder on the depletion band. Its identity as a separate transition is established by observing the region between 500 nm and 600 nm as the phase and modulation frequency is changed, when it is observed that the shape of the absorption band in the 510 nm region changes considerably. More convincing proof comes from polarisation studies which show a sudden drop in the polarisation in the region of 510 nm, suggesting the presence of a transient in antiphase to the 570 nm transient. Moreover, if the 570 nm band is phased out to zero, the 510 nm shoulder is resolved as a distinct band.

Polarisation studies showed that the three major bands are polarised. The bands at 570 nm and 660 nm have polarisations of 0.31 and about 0.25 respectively (Polarisation is defined here as $p = [(I/I_{\perp}) - 1] / [(I/I_{\perp}) + 2]$). This suggests that the pigment is in a fairly viscous environment. The band at 410 nm at first seemed to give an anomalous result, until it was realised that the parallel and perpendicular components of the polarisation at this wavelength are not in phase, as is shown in Fig 2. As a consequence the polarisation obtained varies with the phase setting and the modulation frequency. This indicates the presence of at least two species absorbing at 410 nm with slightly different polarisations and lifetimes.

Attempts to estimate lifetimes using the phase shift proved

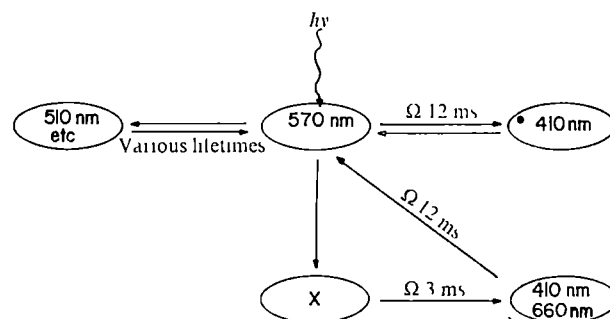


Fig 3 Schematic diagram of kinetic processes. The two complexes absorbing at 410 nm may be conformers.

difficult since the apparent values measured varied with modulation frequency. This shows that the kinetics are not first order. The following facts have however been established. The 410 nm and 660 nm bands are in exact phase at low modulation frequency (5 Hz) with an apparent lifetime of about 15 ms, about 1 ms longer than the 570 complex. At high modulation frequency (108 Hz), the 660 nm band has an apparent lifetime of 1 or 2 ms whereas both the 570 and 410 nm bands still have long apparent lifetimes of about 12 ms, that of the 410 nm band again being slightly longer. In comparison the 510 nm transient is very short lived indeed, with a lifetime of well under 1 ms.

A flash photolysis study showed that both the depletion at 570 nm and the transient at 410 nm have lifetimes of the order of 15 ms, but the kinetics are indeed far from first order. It would appear that the depletion band also has several shorter-lived components, one major one having a lifetime of about 3 ms. The 660 nm transient passes through a well-defined cycle, rising with a lifetime of about 3 ms and decaying with a lifetime of about 12 ms.

A complete explanation of the kinetic observations is not possible at this stage, but a number of salient points emerge. First, the 410 nm band represents the primary phototransient in that the majority of molecules not in the ground state would be found in that state. Second, it seems clear that there are at least two species absorbing at 410 nm, most probably conformers with different polarisations and lifetimes. Furthermore, there is a direct kinetic link between the decay of the 660 nm transient and of the longer-lived 410 nm transient as judged from the phase relationship between them. Apparently at least three pathways exist through which the excited pigment can pass. One pathway renews the pigment rather rapidly, as shown by the observation that the phase of the 570 nm species is always slightly ahead of that of the 410 nm species at both high and low chopping frequencies, one renews it through the 410 nm transient, while the longest pathway involves the 660 nm transient. It seems certain that part of the absorption at 410 nm is associated with the transient which absorbs at 660 nm. This would account for their being in phase at the low modulation frequency. Much of the absorption at 410 nm, however, belongs to a slightly shorter-lived species or at least to a species which decays earlier than the one associated with the 660 nm absorption.

A scheme which fits these observations is shown in Fig 3. On absorption of light by the pigment at 570 nm there is a very speedy relaxation on the part of the excitation, reforming the pigment. This is probably a trivial process of relaxation of the excited triplet-state of the original pigment. At the same time there is a quick conversion of the original pigment to the primary transient absorbing at 410 nm which relaxes slowly back to the original pigment. There is another process which over a period of about 3 ms generates the transient absorbing at 660 nm with its associated absorption at 410 nm. This also decays with a long lifetime of about 12 ms to the original pigment.

The phenomena described above were also observed in basal salt⁵, which made little difference to the spectra above 400 nm.

All samples studied were deoxygenated by purging with argon and sealing, in these conditions they were stable over periods of several weeks at room temperature. The addition of valinomycin in excess had only one effect: the 570 nm polarisation increased by about 10%, but it was found that the lifetime of this transient as measured at the 108 Hz modulation frequency decreased to a similar extent. Evidently the valinomycin quenches some of the shorter-lived species such as the triplet-state, allowing less time for rotational relaxation.

The existence of a conformational change of the 410 nm species may provide a mechanism for the proton pump, in which case the species X shown in Fig. 3 must be the original 410 nm species itself.

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Inhibition of adenyl cyclase by an exotoxin of *Bacillus thuringiensis*

CERTAIN strains of the insect pathogen *Bacillus thuringiensis* produce a heat-stable exotoxin which is toxic to species from several orders of insects^{1,2} and also affects mammals³⁻⁵. The exotoxin inhibits ecdysone-stimulated insect RNA polymerases of mice both *in vivo*⁶ and *in vitro*⁷.

RNA polymerase catalyses nucleophilic attack by an alcohol group on the α -phosphorus of ribonucleoside triphosphates. Adenyl cyclase also belongs to this functional group of enzymes and in the studies reported here the exotoxin has been shown to be a competitive inhibitor of adenyl cyclase, to inhibit both fluoride and hormonal stimulation of this enzyme activity and to block both adrenocorticotrophic hormone (ACTH)-induced increases in adrenal cyclic adenosine monophosphate (cyclic AMP) and adrenal steroidogenesis.

The effects of exotoxin on pigeon erythrocyte adenyl cyclase were studied first. In intact avian erythrocytes, the activity of this enzyme is increased by adrenaline⁸, while in resuspended haemolysed erythrocyte preparations ('ghosts') adenyl cyclase activity is increased by sodium fluoride⁹. Figure 1 shows that the increased adenyl cyclase activity produced by sodium fluoride in 'ghosts', prepared from adult feral pigeons was markedly inhibited by exotoxin. Exotoxin also inhibited the increased adenyl

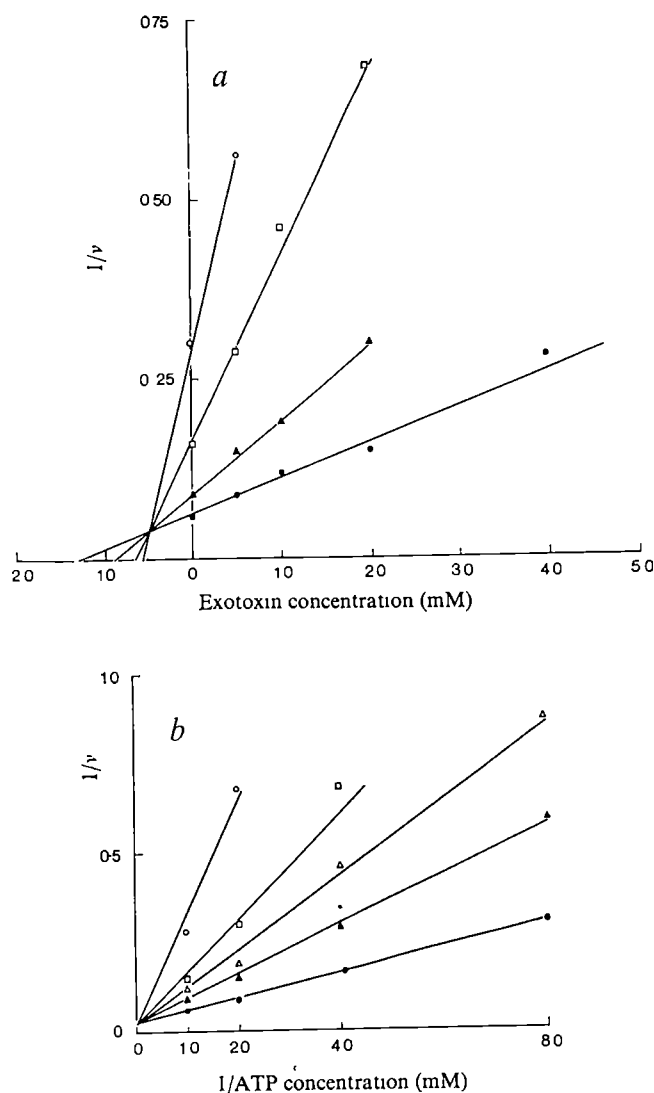


Fig. 1 Inhibition of pigeon erythrocyte adenyl cyclase by exotoxin. To combine results from several experiments, results are expressed as cyclic AMP produced as a percentage of uninhibited adenyl cyclase activity. ○, Erythrocyte 'ghosts' stimulated by sodium fluoride 1×10^{-2} M, ●, intact erythrocytes stimulated by adrenaline 1×10^{-6} M, △, unstimulated level of cyclic AMP production by 200 μ l erythrocyte 'ghosts' suspensions, mean 1.4 ng min^{-1} (range $0.6\text{--}3.0 \text{ ng min}^{-1}$), ▲, unstimulated level of cyclic AMP production by 200 μ l intact erythrocyte suspension, mean 2.2 ng min^{-1} (range $1.4\text{--}3.0 \text{ ng min}^{-1}$). Adult feral pigeons were anaesthetised with pentobarbitone (4 mg per 100 g) injected intramuscularly. Blood was drawn directly from the heart into a heparinised syringe, haemolysed 'ghosts'¹⁰ were prepared from washed erythrocytes⁸. Incubations were performed at 37°C with shaking under 95% $\text{O}_2 + 5\% \text{CO}_2$. The incubation mixture consisted of 200 μ l of the intact or haemolysed erythrocyte suspensions, and ATP (2×10^{-3} M) was added to 'ghost' incubations. A solution of the sodium salt of the exotoxin (5 mg ml^{-1}) was added in increasing volumes, and appropriate volumes of the modified Krebs bicarbonate buffer⁸ containing 6 mM theophylline (in which all added substances were also dissolved) were added to give a final incubation volume of 2 ml. After preincubation of the erythrocyte suspensions for 3 min with exotoxin, adrenaline (final concentration 10^{-6} M) or sodium fluoride (final concentration 10^{-2} M) was added and the incubation continued for a further 5 min. The reactions were terminated by the addition of trichloroacetic acid (TCA) to give a final concentration of 5% (w/v) and the resulting precipitate was removed by centrifugation. The TCA was extracted from the supernatant by shaking with 3 volumes of ether three times. The cyclic AMP in the supernatant was then measured by saturation analysis using the bovine adrenal cyclic AMP binding protein¹¹.

cyclase activity produced by adrenaline in intact erythrocytes, although at low exotoxin concentrations there appeared to be slightly increased cyclic AMP production.

Cyclic AMP is thought to act as the intracellular mediator of the action of many hormones¹². In the adrenal cortex, ACTH stimulates adenylyl cyclase, adrenocortical cell concentrations of cyclic AMP rise, and, by a sequence of reactions not yet fully understood, increased amounts of cholesterol are converted to pregnenolone and increased steroidogenesis ensues. We have studied the effect of exotoxin on this system. Table 1 shows that ACTH produced a 38% increase in cyclic AMP content of the adrenal glands and increased 11-hydroxycorticoid production to 176% of control levels. In the presence of exotoxin, however, ACTH did not produce a significant rise in either cyclic AMP or 11-hydroxycorticoids.

To ensure that exotoxin was not interfering with corticosteroid production independently of its effects on cyclic AMP production, further incubations were performed in which, instead of adding ACTH, cyclic AMP itself was added (final concentration 5×10^{-3} M). This led to a 157% increase in 11-hydroxycorticoid production (Table 1) which was not significantly altered by exotoxin.

These results suggest that exotoxin inhibits the increased activity of adrenocortical adenylyl cyclase produced by ACTH and, therefore, prevents the ACTH-induced rise in adrenocortical cyclic AMP concentrations, concomitantly reducing ACTH-stimulated steroidogenesis. Since cyclic AMP-induced steroidogenesis is not inhibited by exotoxin, other effects of the exotoxin in the mechanisms mediating steroidogenesis distant to the point of action of cyclic AMP were excluded.

The mechanism by which exotoxin inhibits adenylyl cyclase activity was studied in a rat brain preparation. Figure 2 shows that exotoxin competitively inhibits adenylyl cyclase in this preparation with a K_i of 0.5 mM. The K_m for ATP in this preparation was 1.1 mM.

The ability of exotoxin to compete with ATP as a substrate for adenylyl cyclase is probably a property of its structural similarity to the natural substrate. Available evidence suggests that exotoxin differs from ATP in that the 5' position of ribose is substituted with a phospho-allomucyl glucoside rather than a triphosphate^{2,15}. Although a synthetic analogue of ATP, α, β -methylene ATP, has recently been shown to inhibit adenylyl cyclase activity in mammalian liver and fat cell membrane preparations¹⁶, inhibition by other naturally occurring ATP analogues has not previously been reported. 3-Deoxy ATP, isolated from culture filtrates

of the moulds *Cordiceps militans*¹⁷ and *Aspergillus nidulans*¹⁸, inhibits RNA polymerase¹⁹ and seems worthy of study with respect to adenylyl cyclase.

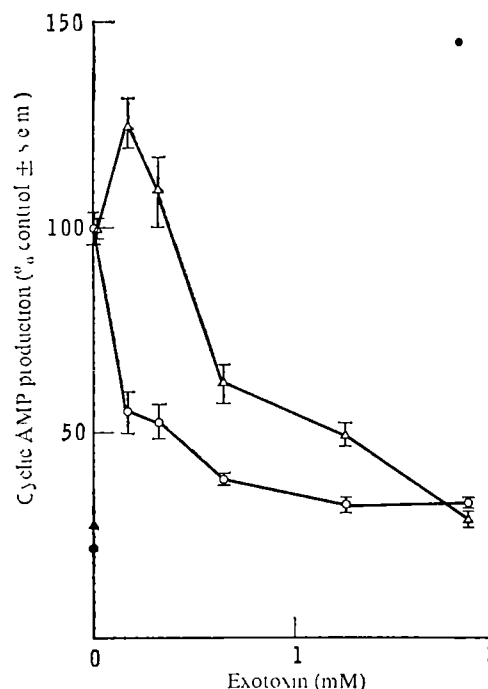


Fig 2 Inhibition of rat brain adenylyl cyclase by exotoxin. a, Effects of altering exotoxin concentration at fixed ATP concentration: ●, No exotoxin; ▲, 0.5 mM; △, 1.0 mM; □, 2.0 mM; ○, 4.0 mM. b, Effects of altering ATP concentration at fixed exotoxin concentration: ATP added: ●, 1.0 mM; ▲, 0.5 mM; □, 0.25 mM; ○, 0.125 mM. v is expressed as nmol cyclic AMP produced per min by the crude mitochondrial fraction derived from 1g brain.

Male Wistar rats (200 g) were decapitated by guillotine, the brain was homogenised in 0.32 M sucrose and a crude mitochondrial fraction (P_2) was prepared¹⁴. The P_2 pellet was resuspended in 10 volumes of 0.3 M Tris-HCl buffer pH 7.4 containing 8 mM theophylline and 3 mM magnesium (v/w original brain wet weight). This suspension was then ultrasonicated for 5 min using a DAWE 1130 Soniprobe producing 75 W at 20 KHz. This mitochondrial preparation (50 μ l) was preincubated at 30°C with sodium fluoride (10 mM) in the presence of exotoxin and an appropriate volume of Tris-HCl-theophylline-magnesium buffer to give a final volume of 200 μ l. After 5 min, ATP was added and the incubation continued for a further 15 min. The reaction was stopped by boiling for 3 min, the precipitate removed by centrifugation and the cyclic AMP content of the supernatant measured.

Table 1 Effects of exotoxin on 11-hydroxycorticoid and cyclic AMP content in rat adrenal quarters*

Additions	11-Hydroxycorticoid content (μ g 100 mg ⁻¹ adrenal gland)		Cyclic AMP content (ng 100 mg ⁻¹ adrenal gland)	
	Mean	s.e.m.	Mean	s.e.m.
None	7.83	0.86	84.1	4.68
ACTH	13.75†	1.4	115.7§	5.51
ACTH + exotoxin	7.83†	0.68	86.73§	6.51
Cyclic AMP	12.28‡	1.73		
Cyclic AMP + exotoxin	11.85‡	1.6		

* Combined results of ten experiments, † difference significant at $P < 0.01$, ‡ difference not significant, § difference significant at $P < 0.01$.

To achieve basal levels of steroidogenesis and cyclic AMP production adult male Wistar rats (150 g) were anaesthetised with pentobarbitone (6 mg per 100 g) injected intramuscularly and decapitated by guillotine, while unconscious, 1 h later. Quartered adrenal glands were preincubated at 37°C for 90 min in Krebs-Ringer bicarbonate pH 7.4 (1.5 ml) containing glucose 100 mg per 100 ml (KRBG), under 95% $O_2 \times 5\%$ CO_2 . The glands were then removed, blotted dry, weighed and then incubated in 2.5 ml of fresh KRBG under 95% $O_2 \times 5\%$ CO_2 in the presence or absence of 6 mM exotoxin. After 15 min, ACTH (Organon) was added (final concentration 2 U ml⁻¹) and the incubation continued for 60 min. The reaction was then terminated by rapid freezing of the medium and glands in solid CO_2 and acetone. After thawing, the glands were homogenised in the incubation medium. TCA (final concentration 5% w/v) was added to 0.5 ml of the homogenate and the protein precipitate removed by centrifugation. TCA was extracted from the supernatant and the cyclic AMP measured as described in the legend to Fig 1. In addition, 0.5 ml of the homogenate was shaken with 4 ml dichloromethane and the 11-hydroxycorticoid content of the dichloromethane extract measured by fluorimetry¹³.

Bacillus thuringiensis exotoxin appears to be a relatively specific competitive inhibitor of adenylyl cyclase and may prove to be a useful tool in the investigation of the role of adenylyl cyclase and cyclic AMP in various systems. Perhaps the ability of this exotoxin, and other potential adenylyl cyclase inhibitors, to block a cellular physiological response to a hormone could be added to the existing criteria for the involvement of cyclic AMP in hormone actions¹²

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Size and DNA content of purified *E. coli* nucleoids observed by fluorescence microscopy

PREVIOUS studies of the physico-chemical properties of DNA as isolated in condensed bacterial chromosomes suggested that the DNA was packaged in particles with dimensions similar to the intracellular nucleoid¹⁻³. This finding, if confirmed, is of significance for it indicates that current methods for isolating the condensed chromosomes preserve the conformational organisation of the DNA at least to the extent that the gross dimensions of the nucleoid are maintained.

Here we demonstrate that the isolated folded chromosomes visualised by fluorescence microscopy have dimen-

sions very similar to those of the intracellular nucleoid. This method also enabled us to determine the DNA content of the nucleoid. We found that the isolated nucleoids contain on average $15 \pm 2 \times 10^{-9}$ μ g of DNA or 3.6 ± 0.5 genome equivalents when doublet, replicating nucleoids were scored as a single structure.

The membrane-free nucleoids were isolated as previously described from *Escherichia coli* strain D-10³. The procedure for fluorescence microscopy was that of Laurent *et al*⁴ who used ethidium bromide as a fluorescent probe to visualise kinetoplast DNA.

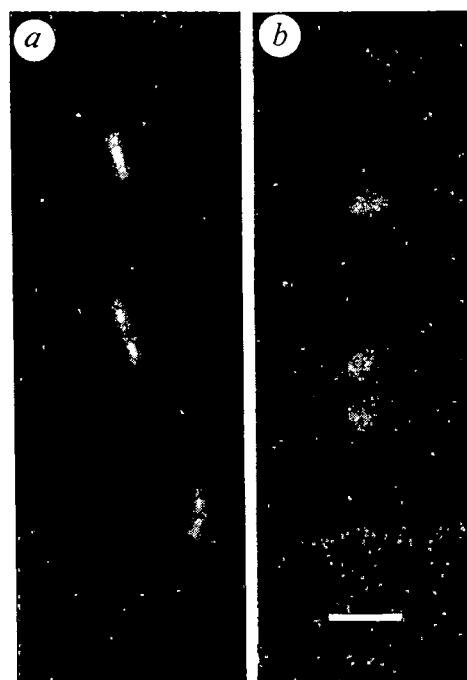


Fig 1 Fluorescence micrographs of the nucleoid of *E. coli*. Cells in exponential growth suspended in growth medium containing $20 \mu\text{g ml}^{-1}$ ethidium bromide. The nucleoid can be seen as the brighter body occupying only part of the cell volume. *b*, Membrane-free isolated nucleoids which had been fixed with formaldehyde and suspended in a solution containing 0.01 M sodium phosphate (pH 7.8), 1.0 M NaCl, 1 mM β -mercaptoethanol, 20% sucrose, 1 mM trisodium citrate and $20 \mu\text{g ml}^{-1}$ ethidium bromide. Fixation was accomplished by mixing sucrose gradient fractions containing the isolated nucleoids with formaldehyde to a final concentration of 0.4% v/v. If Tris-EDTA buffer was present Na phosphate buffer was added to 0.05 M final concentration before the formaldehyde. After incubating for 10 min at 20°C , the fixed nucleoids were resedimented through 30% sucrose on to a self of saturated CsCl solution (45 min at 25,000 r.p.m., 4°C in an SW50.1 rotor). A Leitz Orthoplan microscope with an HB 200 mercury lamp was used with a BG 12 excitation filter and a K 510 nm suppression filter. Photographs were recorded on trix-X ASA 400 film. Scale bar represents 5 μm .

Figure 1a shows a fluorescence micrograph of exponentially growing cells after addition of ethidium bromide. The high spot of intensity is the nucleoid which appears to nearly fill the width of the bacterium but not its length. At the same magnification, the isolated nucleoids (Fig 1b) appear very close in size to the intracellular nucleoid. They range in size from 0.5 to 1.5 μm . Before fluorescence microscopy the isolated nucleoids were fixed by reaction with formaldehyde⁵. Unfixed nucleoids appeared very similar to those shown in Fig 1, however, they were less stable to the conditions of fluorescence microscopy and they gradually expanded and became more diffuse during the microscopy (our unpublished observations). Such changes were not observed with the fixed particles. The fluorescence intensity of the isolated nucleoids is reduced relative to nucleoids

Table 1 Determination of DNA content per nucleoid

Nucleoid concentration per ml ($\times 10^{-6}$)			Concentration of radioactivity in nucleoids c p m ml $^{-1}$ ($\times 10^{-5}$)	Specific Activity of DNA c p m μ g $^{-1}$ ($\times 10^{-5}$)	Average μ g DNA per nucleoid ($\times 10^{-9}$)		Average genome equivalents per nucleoid	
Singles (S)	Doubles (D)	Total (S + D)			S + 2D	S + D	S + 2D	S + D
24 \pm 6	52 \pm 4	76 \pm 10	50	43	91 \pm 10	15 \pm 2	22 \pm 0.2	36 \pm 0.5
					94 \pm 10	15 \pm 2	22 \pm 0.2	36 \pm 0.5

A culture of cells (30 ml) was labelled for 20 min with 100 μ C of 3 H-thymidine (55 Ci mmol $^{-1}$) just before collection of the cells. Generation time of the cells was 29 min. Nucleoids were isolated from a portion of the culture and DNA was purified from the remainder. The method for DNA purification included lysis with lysozyme and sodium dodecyl sulphate, repeated cycles of phenol extraction, three cycles of RNase treatment interspersed with ethanol precipitations and resuspension, and passage through a BioRad P-100 column. The specific radioactivity of the DNA is listed above. The nucleoid concentration in the preparation was determined in a Petroff-Hausser chamber as described in Fig. 1. Both single (S) and double nucleoids (D) were scored. The first line of the Table shows the result of a single measurement averaged over 304 nucleoids. The second line is an average of all our data over 2,379 nucleoids. The calculated DNA content per nucleoid and the number of genome equivalents per nucleoid are tabulated above assuming, on the one hand, that there are two nucleoids per doublet and, on the other hand, that there is one nucleoid per doublet. Radioactivity was counted in a Packard liquid scintillation system, it was determined that 3 H counting efficiencies of the purified DNA and purified nucleoids were the same and hence corrections were not required.

in situ when examined as in Fig. 1. This reduction is at least partially attributable to the higher salt concentrations added to stabilise the purified nucleoids. These salt concentrations are known to increase the dissociation coefficient of ethidium bromide bound to DNA⁵.

When ethidium bromide binds to the DNA of isolated nucleoids it can affect the amount of supercoiling in the structure and thereby alter the state of condensation of the DNA^{2,7}. However, as indicated from previously published hydrodynamic properties of the nucleoids^{2,7}, the conditions and concentration of ethidium bromide used in the present work should not significantly influence the final dimensions of the nucleoids. We have also demonstrated in recent work (unpublished work) that the sedimentation rate of fixed nucleoids as shown in Fig. 2 changes by less than 10% in the presence of these concentrations of ethidium bromide.

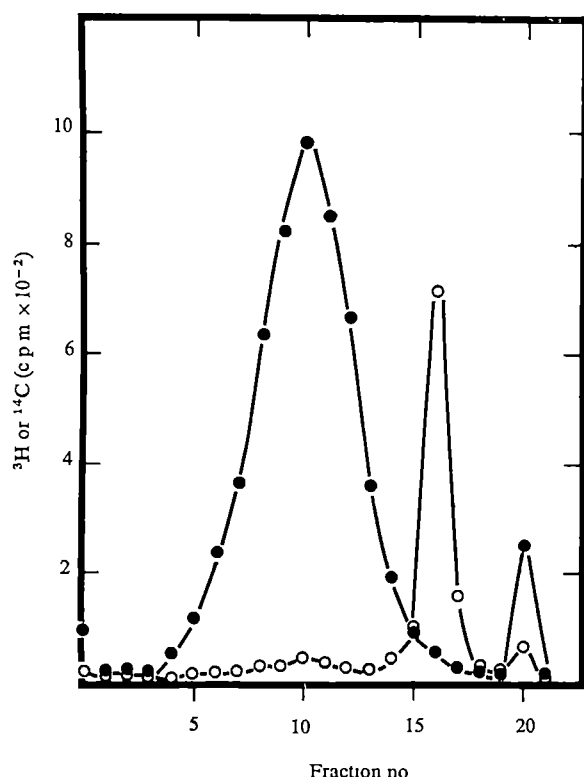


Fig. 2 Sedimentation of fixed nucleoids. After completing the observations described in Fig. 1 and Table 1, an aliquot of the same preparation of fixed nucleoids was diluted, 14 C-T4 phage added as a sedimentation marker (1,025S) and sedimented for 30 min at 4°C and 17,000 r.p.m. through a 10–30% w/v sucrose gradient containing 0.01 M Na phosphate (pH 7.8), 1 mM trisodium citrate and 1.0 M NaCl. ○ 14 C-T4 phage, ● 3 H-DNA in nucleoids.

Rapidly growing cells, such as those observed in this study, frequently contain a pair of nucleoids. When this occurs the two bodies usually seem to be separated towards the opposite poles of the cell (see ref. 8). We also observed a significant number of doublets in the preparations of purified nucleoids (Fig. 1b and Table 1). These were not generated by the fixation treatment since doubles were observed at about the same frequency in unfixed preparations of nucleoids. Resedimentation of the fixed nucleoids confirms the absence of any aggregation as well as reproducing the 1,600S average sedimentation rate of unfixed nucleoids relative to a T4 phage marker (Fig. 2). From these results, it seems likely that nucleoid pairs in the cells may actually be connected and that these connections are preserved during the isolation. Possibly the nucleoids that seem to be separated yet occur in doublets have not yet completed their division.

The concentration of nucleoids in a preparation can be measured conveniently using fluorescence microscopy techniques. From this approach we were able to determine the average DNA content of the isolated fixed nucleoids (Table 1). Depending on whether the double nucleoids were scored as a single body or two, the calculations show that on average the isolated nucleoids are made up of respectively, 3.6 or 2.2 genome equivalents of DNA (A genome equivalent of DNA is the mass of DNA corresponding to a single, non-replicating chromosome). When doublets are counted as a single nucleoid, the DNA content per nucleoid agrees closely with the cellular DNA content of *E. coli* cells grown under similar conditions^{9,10}. This finding offers further support to the above proposal that each of the doublets observed in our preparations was associated as a pair of nucleoids in a single cell.

In scoring the nucleoids for shape, the distinction between the doublet and singlet was not always clear. It was our impression that there may be a continuum in the degree of separation of the nucleoid halves. With this in mind it should be stressed that the distribution between doublets and singlets described in Table 1 was necessarily subjective.

After completing all of the microscopy measurements described in Fig. 1 and Table 1, an aliquot of the fixed nucleoids was resedimented on a sucrose gradient to assess the extent of unfolding or aggregation of the nucleoids which may have occurred during handling or storage. As Fig. 2 shows, the fixed nucleoids maintained their condensed state and the mean sedimentation rate of the particles was indistinguishable from the rate observed immediately after isolation^{6,7}.

In conclusion, we have shown by direct visualisation of the isolated nucleoids by fluorescence microscopy that the chromosomal DNA is isolated in a structure similar in size and DNA content to the nucleoids observed *in vivo*.

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Polyamines stabilise DNA folds

THE DNA of bacterial cells, gently isolated in the presence of high concentrations of monovalent salts, remains folded in a particle resembling the bacterial nucleoid^{1,2}. The double-helical DNA is condensed by folding¹ and by supercoiling³ and the compact structure is dependent both on counterions in the solvent environment and RNA molecules bound to the DNA¹⁻⁴. In the absence of these stabilising interactions the DNA unfolds and acquires the usual properties of extended

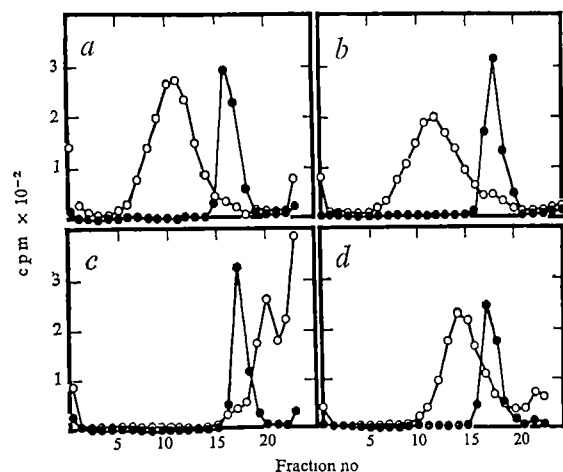


Fig. 1 Sedimentation of folded and unfolded DNA after various periods of storage. Membrane-free nucleoids labelled with ³H-thymidine were isolated on sucrose gradients containing 1.0 M NaCl and other components as described in text, peak fractions were pooled and half of the pool was stored at 4° C. The other half was also stored at 4° C but in the presence of 5 mM spermidine and with NaCl diluted to 0.25 M while holding other components of the gradient buffer constant. At various times aliquots were removed, diluted to approximately the same DNA concentration (0.5-1.5 µg ml⁻¹) and ¹⁴C-labelled T4 phage (1.025S, ref 7) were added as a sedimentation marker. The mixture was layered on a 4.8 ml sucrose gradient (10-30% w/v) containing 0.4 M NaCl, 1.0 mM spermidine and other components as described in the text and centrifuged at 17,000 r.p.m. at 4° C for 30 min in a SW50.1 rotor. The DNA was stored in 1.0 M NaCl for 1 h (a) or 6 d (c) in 0.25 M NaCl plus 5 mM spermidine for 1 h (b) or 6 d (d). ○, ³H-DNA, ●, ¹⁴C-T4 phage

double-helical DNA^{1,3}. The concentrations of monovalent salts required during the isolation of folded DNA are significantly greater than those found in the cell. It is, however, believed that *in vivo* polyamines may be an important counterion neutralising DNA⁵. We have now found that moderate concentrations of the polyamine spermidine greatly stabilise the condensed DNA conformations in isolated nucleoids. In addition to broadening current understanding of the role of counterions in stabilising condensed DNA states, this finding will be of significance to studies in which the stability of the nucleoid *in vitro* is important.

Nucleoids were isolated from *E. coli* strain D-10 as before^{1,2}. We used the membrane-free nucleoid² (1,600S), obtained by incubating the lysate before DNA isolation at 20° C in the presence of 0.5% Brij 58-0.2% deoxycholate. The folded DNA of this particle has less than 10% by weight bound protein, most of which is DNA-dependent RNA polymerase^{1,2,6}. Nascent RNA chains are also bound to the folded DNA, the RNA fraction is 0.4 relative to DNA (w/w) (ref 2). In some cases purification was on sucrose gradients containing 0.01 M Tris (pH 8.1), 1 mM EDTA, 1 mM β-mercaptoethanol and 1.0 M NaCl, in other cases, the gradients contained the same mixture, but with 0.4 M NaCl and 1 mM spermidine. The results described here were similar using both kinds of preparations.

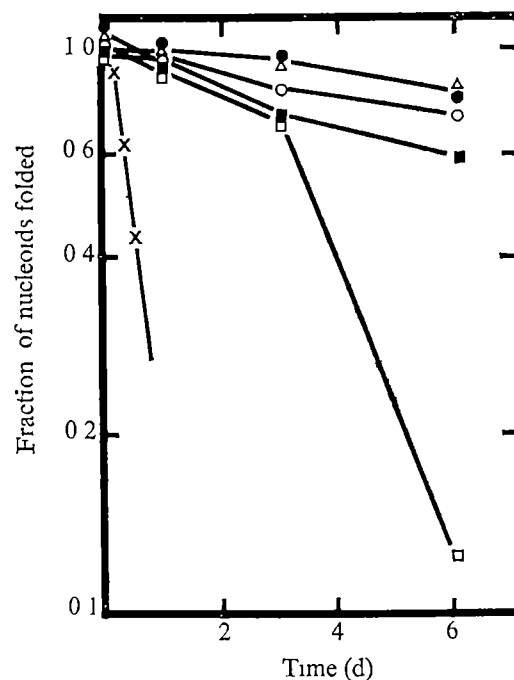


Fig. 2 Stabilisation of folded DNA with spermidine. Isolated nucleoids were stored at 4° C in the presence of varied amounts of spermidine and at times later were sedimented as described in Fig. 1 to estimate the level of unfolding (see text for definition of 'folded'). □, 1.0 M NaCl minus spermidine, x, 0.10 M NaCl minus spermidine, other points had 0.25 M NaCl plus spermidine: 10 mM (●), 5 mM (Δ), 2 mM (○), or 1 mM (■).

As Fig. 1a shows, the folded DNA immediately after isolation sedimented at about 1,600S in a band somewhat broader than that of a homogeneous molecular weight marker. Worcel and Burgi³ have demonstrated that the slight heterogeneity is attributable to the variation in DNA content of nucleoids in different stages of replication. During prolonged storage, with monovalent salts as the stabilising counterions, the DNA gradually unfolded and we observed transition to slower sedimenting structures (Fig. 1c). The evidence that the sedimentation change was derived primarily from conformational alteration of the DNA rather than a reduction of the mass or molecular weight of the structure is essentially the same as reported¹⁻⁴. As in the case of the transition resulting from

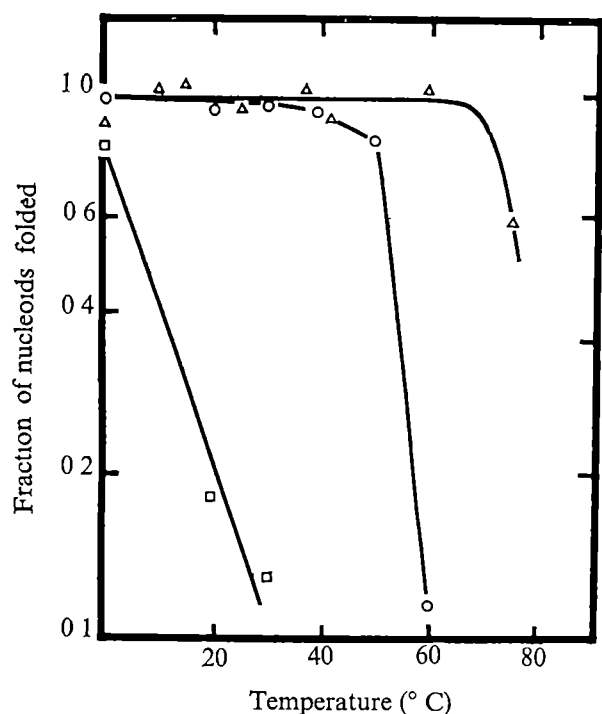


Fig 3 Thermally induced unfolding in the presence or absence of spermidine. Isolated nucleoids were diluted to the same DNA concentration in different solutions so that all components of the storage buffer remained constant (see Fig 1) but the NaCl concentration became 0.1 M and the spermidine concentration was 5 mM (Δ) and 2 mM (\circ) or none was added (\square). The solutions were heated for 5 min at the temperatures shown, chilled to 0°C and sedimented as described in Fig 1 to estimate the level of unfolding.

treatment with reagents which unfold the DNA, there was a simultaneous large increase in relative viscosity and reduction in sedimentation rate. Also, since the mass of the isolated nucleoid is due predominantly to DNA, any dissociation of RNA or protein should by itself not have led to a large reduction in sedimentation rate. Furthermore, our preliminary sedimentation studies (unpublished) of DNA obtained from partially unfolded nucleoids indicate no large change in DNA molecular weight.

To describe the changes that occur in data such as those of Fig 1, we use the following rough criterion. Folded DNA is said to have been unfolded or partially unfolded if, without breaking the DNA, it undergoes transition from a structure sedimenting more rapidly than 1,000S to one sedimenting at less than 1,000S. Until there is a more quantitative understanding of unfolding this criterion will suffice for approximation.

The rate of DNA unfolding during storage was reduced in the presence of spermidine (Fig 1). As Fig 2 shows, unfolding rate was minimal with 5 or 10 mM spermidine and increased with lower concentrations. In the absence of spermidine the nucleoids were not stable for more than a few days, however, they were much more stable in 1.0 M than in 0.1 M NaCl.

Spermidine also stabilised against thermally induced unfolding (Fig 3). In a solvent containing 0.1 M NaCl and 2 mM spermidine the temperature at which half of the nucleoids were partially unfolded appears to be approximately 55°C measured under the conditions described in Fig 3, while it increased to more than 70°C in the presence of 5 mM spermidine. In the same solvent without spermidine the analogous temperature was about 15°C and nearly all the DNA was partially unfolded at 37°C (see Fig 4 for relevant sedimentation profiles). The sharpness of the thermally induced transition suggests that the molecular interactions stabilising folded DNA, like those stabilising the double helix, are cooperative. By comparison the T_M for denaturation of double helical *Escherichia coli* DNA in the same solvent without

spermidine was 85°C and with 5 mM spermidine added it increased to about 90°C.

The sedimentation method used here to observe the unfolding transition, is particularly sensitive to partial unfolding of DNA⁴. Using the criterion described above, DNA which has partially unfolded and sediments at rates less than 1,000S is scored the same as completely unfolded DNA. One would therefore expect that when precise methods are available for quantitating the amount of unfolding, the transition temperatures required to remove half of the DNA folds will be somewhat larger than our estimates.

We occasionally observed an apparent aggregation of isolated nucleoids mediated by spermidine. This was most obvious at the highest spermidine concentrations (above 2 mM) and at the lowest DNA concentrations (less than 1 μ g DNA ml⁻¹). It was manifested by an increased sedimentation rate and increasingly broad sedimentation profile of the nucleoids. In extreme cases at high spermidine concentrations complete precipitation of the nucleoids was observed using the sedimentation conditions described in Fig 1.

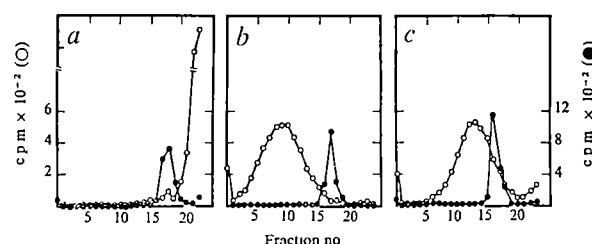


Fig 4 Sedimentation profiles of folded DNA after heating. Three sucrose gradients from the data summarised in Fig 3 are shown: (a) 37°C minus spermidine, (b) 37°C, 2 mM spermidine, (c) 60°C, 5 mM spermidine. \circ , ³H-DNA; \bullet , ¹⁴C-T4 phage.

Polyamines are known to stabilise different nucleic acid structures and protein-nucleic acid complexes. Examples are such interactions with double-helical DNA^{8,9}, viruses¹⁰, ribosomes^{11,12} and triple-stranded nucleic acid structures¹³. Polyamines also stimulate *in vitro* a wide range of different enzymatic reactions (see ref 5 for review). The results described here show that stabilisation of condensed DNA in bacterial chromosomes by spermidine cannot be replaced even by very high concentrations of monovalent salt. It seems possible that the bifunctional character of spermidine could permit a linking interaction with two closely packed regions of a DNA double helix or at the site of an RNA-DNA association.

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Position of regularly spaced single-stranded regions relative to 5-bromodeoxyuridine-sensitive sites in sea urchin morula DNA

5-BROMODEOXYURIDINE (BUdR) inhibits cell differentiation and embryogenesis and yet has little effect on cell division or metabolism¹. When sea urchin embryos are grown in the presence of BUdR, pre-gastrula DNA accumulates as 30–60S duplex pieces². We have now found that naturally occurring, regularly spaced, single-stranded regions in morula DNA³ are the sites at which BUdR-induced breakage occurs, resulting in production of these 30–60S low molecular weight DNA pieces. The DNA pieces from morulae grown in BUdR are approximately half the length of DNA molecules from control embryos grown in thymidine (TdR).

In the labelled DNA preparations used at least 85% of the isolated radioactivity is covalently bonded, as determined by enzymatic and alkaline hydrolysis². Also, ³²P-DNA should represent only nuclear sequences since mitochondrial DNA is not synthesised until after the morula stage^{4,5}. Both of these points have been confirmed by CsCl buoyant density centrifugation of morula ³²P-DNA. Based on density centrifugation, we calculated that approximately 41% of the TdR residues in ³²P-DNA isolated from morulae grown in the presence of BUdR (50 µg ml⁻¹) are BUdR-substituted (S T C, unpublished data).

The effect of *Aspergillus oryzae* single-strand specific S₁ nuclease⁶ on the sedimentation profile of morula DNA was examined on neutral sucrose gradients. DNA was isolated from control culture embryos grown from fertilisation in the presence of 50 µg ml⁻¹ TdR and ³²P. The DNA was mixed with a reaction buffer optimised for S₁ nuclease activity and divided into two equal portions. S₁ nuclease was added to one half while the other was a control without enzyme. Both were centrifuged through sucrose gradients as described in Fig 1. The bulk of labelled morula DNA sedimented as a single zone (Fig 1a) at approximately 51.3S (Table 1). When previously treated with S₁ nuclease, this DNA sedimented more slowly with a peak value of 39.8S (Fig 1b, Table 1).

The effect of S₁ nuclease treatment on the sedimentation profile of morula ³²P-DNA, isolated from embryos grown in the presence of BUdR, was also examined on neutral sucrose gradients. The major portion of this DNA sediments at about 42.7S (Fig 2a, Table 1). After S₁ nuclease treatment, this BUdR-substituted DNA is reduced to a predominant size-class having a peak sedimentation value of 31.1S (Fig 2b, Table 1). Thus, the bulk of the BUdR-substituted duplex DNA sedimented more slowly than the corresponding DNA

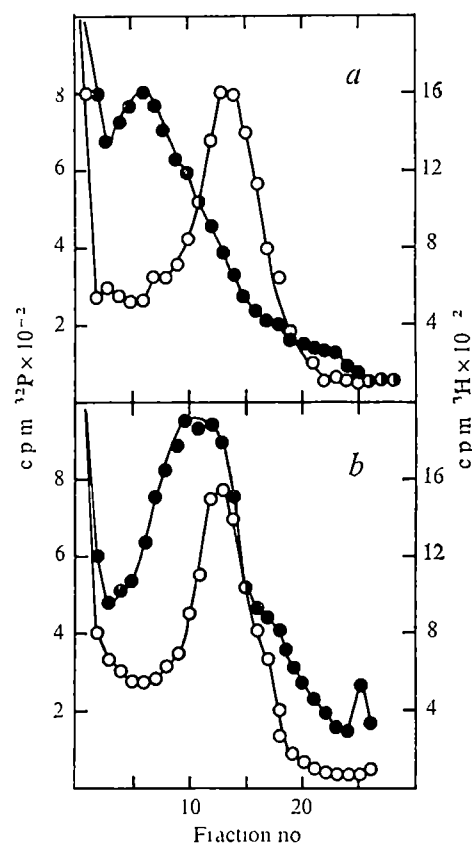


Fig 1 Effect of S₁ nuclease on neutral sucrose sedimentation profiles of morula ³²P-DNA isolated from embryos grown in the presence of TdR. (a) ³²P-DNA incubated without addition of S₁ nuclease, (b) ³²P-DNA incubated with S₁ nuclease. *Strongylocentrotus purpuratus* embryos were cultured¹⁴ in artificial seawater¹⁵. TdR was added to 50 µg ml⁻¹ and ³²P was added to 0.5 µCi ml⁻¹ at 5 and 10 min, respectively, after fertilisation and were both present throughout the growth period. Development was terminated and DNA extracted as previously described². 200 µg of ³²P-DNA (specific activity, 650 c.p.m. µg⁻¹) was mixed with a buffer providing final concentrations of 0.15 M NaCl, 0.03 M sodium acetate, pH 4.5, and 10⁻⁴ M ZnSO₄. 25 U (ref 16) of S₁ nuclease were mixed with 100 µg of ³²P-DNA while the remaining 100 µg of DNA was the control without S₁ nuclease. Both samples were incubated at 40° C for 45 min and the enzymatic reaction was terminated by chilling the samples and adding 10⁻¹ volume of 0.5 M EDTA. 50 µg of ³²P-DNA from each reaction mixture was combined with 10 µg ³H-TdR-labelled φ80 phage DNA, brought to a final volume of 1 ml with 0.15 M NaCl, 0.015 M sodium citrate and layered on to a 30 ml 5–20% linear sucrose gradient made in 1 M NaCl, 0.02 M Tris-HCl, pH 7.3, and 1 mM EDTA. Centrifugation was in a Beckman SW 25.1 rotor at 24,000 r.p.m. for 7 h at 20° C. Approximately 1.2 ml fractions were collected from the bottom of the centrifuge tubes and the DNA precipitated by the addition of 2 ml of 10% trichloroacetic acid and 50 µg bovine serum albumin. Samples were collected on Whatman GF/A filters and counted in a toluene-based scintillation fluid. ○, ³H, ●, ³²P.

Table 1 Effect of S₁ nuclease treatment on sucrose gradient sedimentation characteristics of ³²P-DNAs isolated from sea urchin morulae grown in the presence of either TdR or BUdR

	S ₁ treated	Neutral gradients			Alkaline gradients		
		Peak S _{20,w}	Molecular weight × 10 ⁻⁶	Base pairs × 10 ⁻³	Peak S _{20,w}	Molecular weight × 10 ⁻⁶	Bases × 10 ⁻³
TdR	—	51.3	97.7	148.1	38.9	14.8	44.8
	+	39.8	46.3	70.2	34.8	11.1	33.8
BUdR	—	42.7	57.5	87.1	38.3	14.2	43.0
	+	31.1	23.1	35.0	35.4	11.6	35.2

Neutral sedimentation data were obtained from Figs 1 and 2. Alkaline sedimentation data were obtained by centrifugation of 50 µg of sea urchin ³²P-DNA mixed with 10 µg of ³H-φ80 phage DNA. The DNAs were made 0.2 M NaOH and held at 37° C for 30 min before layering on to 30 ml 5–20% linear sucrose gradients made in 0.1 M NaOH, 0.9 M NaCl, 1 mM EDTA. Conditions of centrifugation were the same as described in Fig 1. Conditions for S₁ nuclease treatment are given in Fig 1. S_{20,w} values were determined by the relative sedimentation distance of ³H-φ80 phage DNA as marker, cosedimented in each gradient. Molecular weight was calculated from S_{20,w} values using the equations of Studier¹². The calculated S_{20,w} value for φ80 phage DNA is 33.4 based on a molecular weight of 28.6 × 10⁶ (ref 13). For neutral sucrose gradients, calculations were based on continuous (gap-free) double-stranded pieces.

isolated from embryos grown in the presence of TdR. In addition, S₁ nuclease treatment reduced the sedimentation rate of the BUdR-substituted DNA even more than the enzyme affected DNA isolated from control embryos.

The effect of S₁ nuclease on sedimentation profiles obtained for morula ³²P-DNA isolated from cultures grown in the presence of TdR or BUdR was examined on alkaline sucrose gradients (Table 1). Non-enzyme-treated DNA, isolated from a control culture, sedimented as a single region of radioactivity at 38.9S. When treated with S₁ nuclease before denaturation, this DNA sedimented as a zone at 34.8S in alkali. ³²P-labelled BUdR-containing DNA had an approximate sedimentation value of 38.3S in alkaline sucrose. An equivalent sample of this latter DNA, treated with S₁ nuclease before denaturation, sedimented as a sharp zone at 35.4S. While the bulk of native BUdR-substituted DNA was approximately half the length of

native DNA isolated from control embryos, alkaline denaturation of either of these DNAs resulted in molecules of approximately equal size and about one-fourth the length of the native DNA from control embryos (Table 1). Digestion of single-stranded regions by S_1 nuclease before denaturation slightly reduced the size of both the resulting single-stranded control and BUdR-substituted DNAs to approximately equal-length pieces (Table 1).

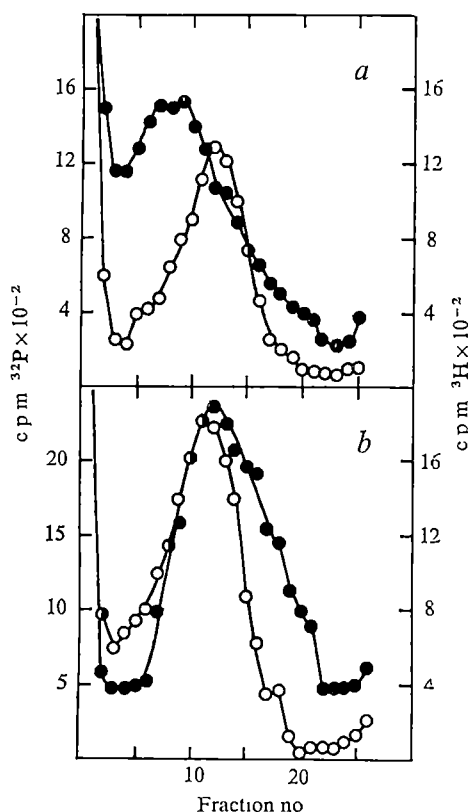


Fig 2 The effect of S_1 nuclease on neutral sucrose sedimentation profiles of morula ^{32}P -DNA isolated from embryos grown in the presence of BUdR. (a) ^{32}P -DNA incubated without the addition of S_1 nuclease, (b) ^{32}P -DNA incubated with S_1 nuclease. Embryos were grown and ^{32}P -DNA extracted as described in Fig 1 except that TdR was replaced by BUdR ($50\text{ }\mu\text{g ml}^{-1}$). $200\text{ }\mu\text{g}$ of ^{32}P -DNA (specific activity, $450\text{ c.p.m. }\mu\text{g}^{-1}$) was mixed with NaCl-sodium acetate- ZnSO_4 , $100\text{ }\mu\text{g}$ of the DNA was treated with S_1 nuclease while the remaining $100\text{ }\mu\text{g}$ was incubated without enzyme. $50\text{ }\mu\text{g}$ of both the control and S_1 nuclease treated ^{32}P -DNAs were mixed separately with $10\text{ }\mu\text{g}$ of ^3H -TdR-labelled $\phi 80$ phage DNA. Conditions for sedimentation and collection of samples were identical to those described in Fig 1. \circ , ^3H ; \bullet , ^{32}P .

A model is presented in Fig 3 for the structure of morula DNA based on the neutral and alkaline sucrose sedimentation data summarised in Table 1. While length determinations of DNA molecules on sucrose gradients are rather imprecise, the proportional molecular lengths corresponding to the peak sedimentation values obtained are in good agreement with the model. Duplex DNA molecules isolated from morula stage embryos are smaller than one would predict for chromosome-length DNA^{7,8} and thus presumably represent subunits. Each subunit of DNA from control (TdR) embryos, represented by L in Fig 3a, must contain a centrally located S_1 nuclease-sensitive site since treatment with the enzyme produces duplex fragments approximately half the length ($1/2\text{L}$ in Fig 3a) of the L molecule. Alkaline denaturation of the original duplex molecule (L) or the duplex fragments generated by S_1 nuclease ($1/2\text{L}$) yields single stranded DNA pieces one-fourth the length ($1/4\text{L}$ in Fig 3a) of the original subunit. Thus, while each L molecule contains one centrally located S_1 nuclease site, each $1/2\text{L}$ molecule contains one centrally located alkali-sensitive site. The S_1 nuclease and alkali-labile sites in native DNA must

each consist of an interruption in one strand, followed closely by an interruption in the other strand of the duplex molecule. Thus, native DNA is held together at an S_1 site or an alkali-sensitive site by hydrogen bonding of the resulting overlapping cohesive single-stranded regions. If the one S_1 site and the two alkali-sensitive sites on an L molecule were the result of discontinuities in only one strand, denaturation would produce one L and four $1/4\text{L}$ single-stranded molecules. A single peak of sedimentation under alkaline conditions supports the assertion that there are discontinuities in both strands of the native L molecule. Perhaps the difference between an S_1 nuclease site and an alkali-labile site in morula DNA is that the S_1 site is a pair of adjacent gaps while the alkali-labile site is a pair of adjacent nicks. Gaps are sensitive to both S_1 nuclease and denaturation while nicks are insensitive to the enzymatic treatment under the conditions used.

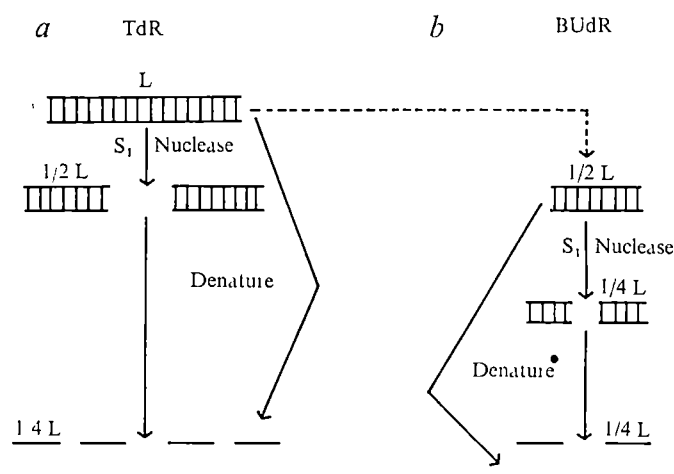


Fig 3 A model of the relationship of regularly spaced S_1 nuclease sites and BUdR-sensitive sites in sea urchin morula DNA.

In previous work³, the average size of morula ^3H -DNA was reported to be somewhat less than that of the equivalent ^{32}P -DNA measured here. Since ^3H -radiation damage to DNA^{9,10} occurs at a faster rate than ^{32}P -radiation damage¹¹ and since the ^{32}P -DNA preparations were used as soon as possible after labelling, we believe that the larger sedimentation values reported here are more accurate.

The duplex DNA molecules isolated from embryos exposed to BUdR are equivalent in length to $1/2\text{L}$ molecules (Fig 3b). Unlike the TdR- $1/2\text{L}$ molecules, however, the BUdR- $1/2\text{L}$ molecules have a central S_1 nuclease sensitive site. Production of BUdR- $1/2\text{L}$ molecules may have resulted from excision *in vivo* of short regions in the L molecules or possibly from a deficiency in joining of $1/2\text{L}$ molecules.

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Deficiency of the Y base in a hepatoma phenylalanine tRNA

THE structures of various tRNAs are of considerable interest as this type of RNA plays a central role in translation of the genetic code and has been shown in bacteria to function in cell regulation^{1,2}. In the search for a possible role of tRNA in regulatory processes in higher organisms, a number of studies have compared chromatographic profiles of tRNA from different tissues, organs, tumours and cell cultures³. These studies have revealed that tumour cells frequently contain tRNA species which differ in their chromatographic mobility from the corresponding tRNAs present in normal adult tissues. Until now, however, no structural basis for these tRNA differences between normal and malignant tissues has been elucidated.

One of the most consistent differences reported for tumour tRNAs involves phenylalanine tRNA (tRNA^{Phe}). Several laboratories have demonstrated by reverse phase column chromatography that certain chemically induced rat hepatomas contain, in addition to the single species of tRNA^{Phe} found in normal rat liver, an early eluting peak not normally found in rat liver^{4,5}. It is known that tRNA^{Phe} of normal eukaryotic cells contains a highly fluorescent and hydrophobic base, called Y, which is located next to the 3'-end of the anticodon⁶. The Y compound represents the most highly modified nucleic acid base characterised so far, consisting of a tricyclic imidazo derivative of guanine to which is usually attached a 4-carbon side chain⁷. In this report we present evidence that the additional species of tRNA^{Phe} found in rat hepatomas differs from normal liver tRNA^{Phe} by the absence of the Y base. Our results constitute the first elucidation of a structural difference between a single species of tRNA from malignant cells compared with the corresponding tRNA from normal cells.

Normal rat liver tRNA was aminoacylated with ¹⁴C-phenylalanine and cochromatographed on a RPC-5 column⁸ with tRNA from Morris hepatoma 7777 aminoacylated with ³H-phenylalanine. Figure 1a shows, in agreement with other

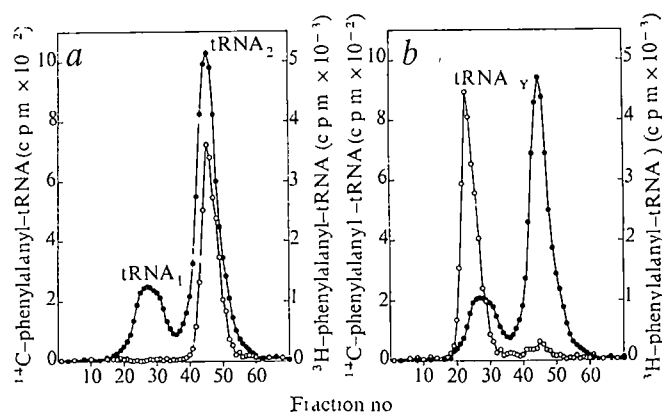


Fig 1 Elution profiles of liver and hepatoma tRNA^{Phe} from RPC-5 columns. *a*, ³H-phenylalanyl-tRNA from Morris hepatoma 7777 cochromatographed with ¹⁴C-phenylalanyl-tRNA from normal rat liver (○). tRNA was prepared essentially according to Fink *et al*¹⁴. Crude aminoacyl-tRNA synthetase preparations were obtained from homogenates of normal liver in medium containing 0.01 M Tris-HCl (pH 7.5), 0.1 M KCl, 5 mM MgCl₂, 0.3 M sucrose and 0.014 M β-mercaptoethanol. After removal of cellular membranes and ribosomes by centrifugation at 105,000g for 2 h, the supernatant was passed over a Sephadex G-50 column, and the void volume containing the enzymes was collected and stored in 50% glycerol at -20°C. Labelled tRNA^{Phe} was prepared by incubating 0.5-1 mg tRNA from liver or Morris hepatoma 7777 with aminoacyl-tRNA synthetase (3.1 mg protein) in a 1 ml reaction mixture containing 10 mM KCl, 10 mM MgCl₂, 4 mM ATP (neutralised), 0.6 mM CTP (neutralised), 0.1 M Tris-HCl (pH 7.3), 0.2 mM each of nineteen amino acids (minus phenylalanine), and either 5 μCi ¹⁴C-phenylalanine (specific activity 460 Ci mol⁻¹), or 100 μCi of ³H-phenylalanine (specific activity 7 Ci mmol⁻¹) for 20 min at 37°C. The labelled tRNA^{Phe} was isolated by applying the entire reaction mixture to a 22 × 22 cm DEAE-cellulose column (DE 52 Whatman). The column was washed with 60 ml of starting buffer (50 mM acetate (pH 4.5), 1 mM MgCl₂, 0.4 mM EDTA), proteins were eluted with 20 ml 0.3 M NaCl in starting buffer, and finally tRNA^{Phe} was eluted with 10 ml 0.6 M NaCl in starting buffer. After precipitation of tRNA^{Phe} with two volumes of ethanol at -20°C, it was dissolved in 0.5 ml of starting buffer and applied to a RPC-5 column⁸. The column (0.7 × 17 cm) was eluted with a 100 ml linear gradient of 0.6-0.7 M NaCl in starting buffer at 250 pound inch⁻². Fractions (1.2 ml) were collected at a flow rate of 2 ml min⁻¹, 0.1 ml aliquots were precipitated with 1 ml 10% trichloroacetic acid at 4°C, filtered on to Millipore filters, dried and counted in a liquid scintillation spectrometer. *b*, ¹⁴C-phenylalanyl-tRNA_Y from liver (○) cochromatographed with ³H-phenylalanyl-tRNA from Morris hepatoma 7777 (●). Liver tRNA (20 A₂₆₀ units) was incubated in 0.5 ml of 0.05 M citrate buffer, (pH 2.9) for 18 h at 37°C, reprecipitated with 2 ml ethanol at -20°C, aminoacylated and processed as described in (a).

results^{4,5}, that rat liver contained only one and hepatoma tRNA two, tRNA^{Phe} species. The major hepatoma peak (tRNA₂^{Phe}) eluted in the same position as rat liver tRNA^{Phe}

Table 1 Poly (U)-directed ribosomal binding of hepatoma ³H-phenylalanyl-tRNAs

tRNA species	³ H-phenylalanyl-tRNA (pmol added)	pmol bound		Δpmol bound
		(-Poly(U))	(+Poly(U))	
Experiment 1				
tRNA ₁ ^{Phe}	11.7	1.9	5.9	4.0
tRNA ₂ ^{Phe}	16.6	3.1	15.4	12.3
Experiment 2				
tRNA ₁ ^{Phe}	7.8	1.4	4.4	3.0
tRNA _{2a} ^{Phe}	7.8	2.7	2.5	-0.2
tRNA _{2b} ^{Phe}	10.4	1.3	8.2	6.9

The ribosomal binding assay was essentially that of Nirenberg and Leder¹³. In experiment 1 the incubation mixture contained in 50 μl 1.3 A₂₆₀ *Escherichia coli* ribosomes, 0.02 M Mg²⁺, 0.1 M Tris-HCl (pH 7.2), 0.05 M KCl, 0.5 μg Poly(U) and ³H-phenylalanyl-tRNA from Morris hepatoma 7777 as indicated. Incubations were carried out at 24°C for 20 min, then diluted with three volumes of the incubation buffer, filtered on to Millipore membrane filters and washed extensively with the same buffer. Radioactivity was measured in a Nuclear Chicago Mark II liquid scintillation spectrometer (1 pmol = 385 c.p.m.).

In experiment 2 the incubation system contained in 100 μl 2.6 A₂₆₀ ribosomes, 1 μg poly(U) and hepatoma ³H-phenylalanyl-tRNAs after acid treatment and separation on RPC-5 columns as shown in Fig 2a and b. Other details were as in experiment 1.

The minor hepatoma peak (tRNA₁^{Phe}) eluted at a lower salt concentration

The earlier elution of tRNA₁^{Phe} suggested that it might lack the very hydrophobic Y base which is present in liver tRNA^{Phe} and hepatoma tRNA₂^{Phe}. This base can be selectively excised from tRNA by mild acid treatment without breaking the polynucleotide chain⁹. tRNA modified in this way (tRNA_{-Y}) can be aminoacylated with phenylalanine, although its codon recognition is impaired^{9,10}. Figure 1b shows the results obtained when rat liver tRNA was incubated at pH 2.9 for 18 h to remove its Y base, aminoacylated with ¹⁴C-phenylalanine and cochromatographed on RPC-5 with hepatoma ³H-phenylalanyl-tRNA (Fig. 1b). After acid treatment, the normal tRNA_{-Y} eluted at a lower NaCl concentration and in a position similar to, but slightly earlier than, hepatoma tRNA₁^{Phe}.

We also isolated hepatoma tRNA₁^{Phe} and tRNA₂^{Phe} on an RPC-5 column. Each was incubated at pH 2.9 for 2 h and rechromatographed separately on RPC-5 columns. The elution position of tRNA₁^{Phe} was unchanged by the acid treatment since the peak appeared at fraction 25 before or after pH 2.9 treatment (compare Figs 1a and 2a). On the other hand, acid treatment of hepatoma tRNA₂^{Phe} (Fig. 2b) resulted in the formation of an earlier eluting fraction (tRNA_{2a}^{Phe}) corresponding to the elution position of normal liver tRNA_{-Y} (Fig. 1b). Acid treatment for 2 rather than 18 h does not completely remove the Y base (compare Figs 1b and 2b). Acid treatment of hepatoma tRNA for 18 h, however, also failed to alter the elution position of hepatoma tRNA₁^{Phe} although it converted 100% of hepatoma tRNA₂^{Phe} to earlier eluting material (separate studies not shown here). These results indicate that hepatoma tRNA₂^{Phe} contains the Y base, but hepatoma tRNA₁^{Phe} does not.

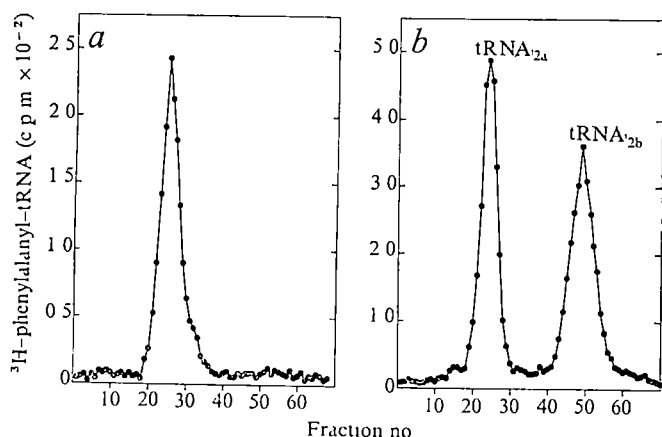


Fig 2 RPC-5 elution profile of acid treated (a) ³H-phenylalanyl-tRNA₁ and (b) ³H-phenylalanyl-tRNA₂ from Morris hepatoma 7777. ³H-phenylalanyl-tRNA₁ or ³H-phenylalanyl-tRNA₂ separated on a RPC-5 column (not shown here but identical to separation as in Fig. 1a) were incubated in 0.05 M citrate buffer (pH 2.9) for 2 h, directly applied to an RPC-5 column and processed as described in the legend to Fig. 1.

Since liver tRNA_{-Y} can accept phenylalanine but is impaired in ribosomal binding^{9,10} we studied poly(U)-directed ribosomal binding of the two species of hepatoma tRNA^{Phe} before and after acid treatment. In agreement with previous studies⁴ we found that before the acid treatment both species were active in this reaction (Table 1, experiment 1). When hepatoma tRNA₂^{Phe} was treated with acid, a portion of the material now eluted in the early region of the RPC-5 column (designated tRNA_{2a}^{Phe}). When this material was assayed, it failed to participate in poly(U)-directed ribosomal binding (Table 1, experiment 2). Similar results were

obtained with normal liver tRNA^{Phe} (data not shown). These findings extend previous studies with yeast tRNA^{Phe} indicating that removal of the Y base leads to loss of codon recognition^{9,10}. On the other hand, we found that acid treatment of hepatoma tRNA₁^{Phe} did not impair its ability to participate in codon recognition (Table 1, experiment 2).

These experiments indicate that hepatoma tRNA₁^{Phe} does not contain the fluorescent and hydrophobic Y base normally present in rat liver tRNA^{Phe}, as its elution position on an RPC-5 column did not change after acid treatment and it retained its binding capacity to ribosomes in the presence of poly(U).

Because excision of the base from normal liver tRNA^{Phe}, and from the major tRNA^{Phe} present in hepatomas, inactivates codon recognition we can conclude that hepatoma tRNA₁^{Phe} is not simply derived from the normal tRNA^{Phe} species as an artefact due to partial excision of the Y base during isolation of hepatoma tRNA. tRNA₁^{Phe} can function in codon recognition because, although it lacks the Y base, it presumably has another base in the position adjacent to the 3' side of its anticodon. From our present data it is not possible to decide whether the tRNA₁^{Phe} species represents the expression of a separate tRNA gene (perhaps a foetal tRNA gene¹⁵) in certain hepatomas or only an immature form of tRNA₂^{Phe} resulting from a failure to complete the synthesis of Y base from its precursor guanosine molecule^{11,12}. Nucleotide sequence studies should resolve this question and these are in progress.

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Primary structure and sidechain interactions of PFL filamentous bacterial virus coat protein

Two structural classes of filamentous bacterial viruses have been identified on the basis of their X-ray diffraction patterns. The class I structure¹ is found for the fd, If1 and IKe strains, while the class II structure² is found for the Pf1 and Xf strains. The two classes differ in the number of protein subunits per turn in the virus helix (4.5 units per turn for class I and 4.4 for class II, with ~ 15 Å pitch), and in the fact that a periodic perturbation of the structure is observed for class I but not for class II. The major coat protein, which comprises about 99% of the virus coat, is largely α -helical^{3,4} with a molecular weight of about 5,000 for all strains investigated². The sequence of the fd coat protein^{4,5} is known. It is 50 residues long, with an acidic N-terminal region, a hydrophobic middle region and a basic C-terminal region. To facilitate the detailed analysis of the class II structure, and to investigate the possibility that the structural differences between class I and class II arise from differences in the coat protein, we have determined the sequence of Pf1 coat protein. This is the first system for which a molecular model of a structural protein has been described in sufficient detail to permit study of the bonding specificity between proteins. Since the α helix is a universal structure, study of the interactions between α helices can be of central importance in many unrelated systems. We have found that α helices are arranged in the virion so that hydrophobic sidechains on each protein subunit can fit into the space between sidechains on neighbouring subunits.

Pf1 virus was grown and purified as described previously², and the coat protein was purified from the virion by phenol extraction and methanol precipitation⁴.

The coat protein of Pf1 consists of 46 amino acid residues. Its amino acid composition is somewhat unusual in that it lacks proline, cysteine, histidine and tryptophan. The sequence was determined by several methods. The first 39 residues from the N terminus were established by automatic sequencing using the JOEL-JAS 47K sequence analyser. The C-terminal sequence was shown to be -Arg-Lys-Ala by carboxypeptidase A and B digestion. Treatment of Pf1 coat protein with cyanogen bromide gave three fragments containing 23, 19 and 4 residues which were separated by gel filtration and paper electrophoresis. Amino acid analysis showed that these fragments accounted for the entire coat protein. It also facilitated the assignment of the 19-residue fragment as the N terminus and the 4-residue fragment as the C terminus. The structure of the 23-residue fragment was determined by automatic sequencing. These results were confirmed by the isolation and sequencing of the five chymotryptic peptides obtained from a digest of the succinylated protein. The results of these determinations showing the location of the cyanogen bromide fragments and chymotryptic peptides, and sequences established by Edman degradation and carboxypeptidase digestion, are shown in Fig 1.

The coat protein sequence of Pf1 is compared with that of fd in Fig 2. The sequences are aligned on the basis of similarities in the predicted secondary structure. With this alignment, the average minimum base change per codon⁶ required to go from the Pf1 to the fd sequence would be 1.24. Although fd and Pf1 might be related by convergent evolution, this seems unlikely in view of the complexity of the virions. If the two viruses are related by divergent evolution, a time scale for evolution⁷ calibrated on minimum base change per codon suggests that the two viruses would share a common ancestor in existence about 3×10^9 yr ago. The similarity² between the A proteins

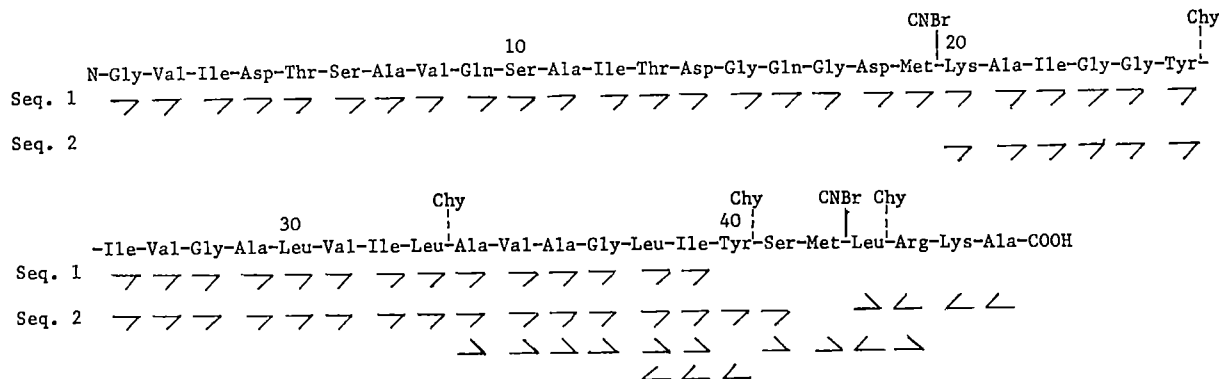


Fig 1 Determination of the Pf1 coat protein sequence. Seq 1 and seq 2 (→) are determinations using the JOEL-JAS 47K sequence analyser, (↔) are sequences determined by dansyl-Edman degradation, and (←) are sequences established by carboxypeptidase A and B digestion. CNBr and Chy indicate sites of cleavage by cyanogen bromide and chymotrypsin, respectively.

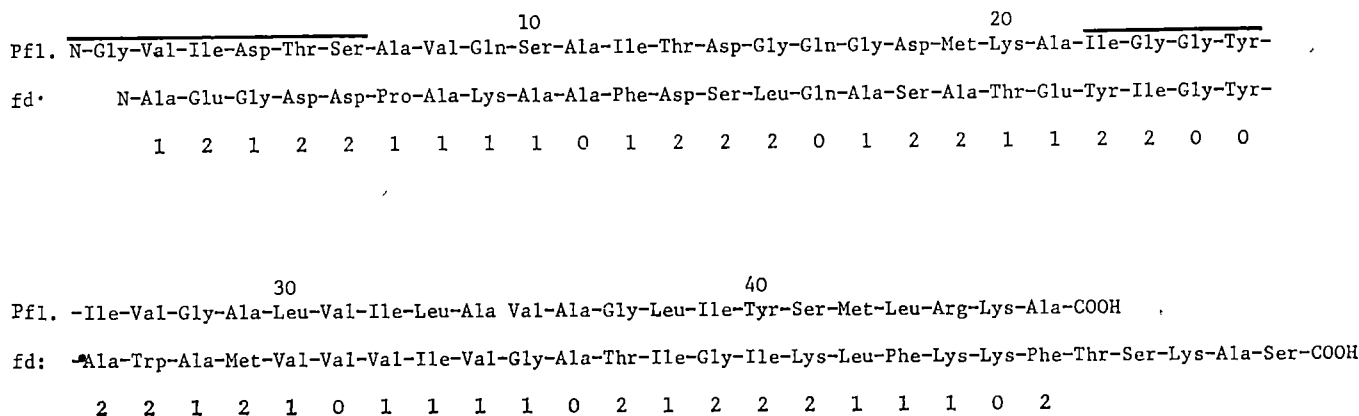


Fig 2 Comparison of the Pf1 and fd coat protein sequences. Sequences were aligned on the basis of the positions of non-helical residues and the positions of basic residues at the C terminus. The regions identified by bars have low probability of α helix⁹. The numbers in the third row indicate the minimum base change per codon required to give the observed amino acid change, taken from Table 2 of ref 6.

of the two highly divergent strains would then suggest that this protein has some fundamental and immutable function in the life cycle of the virus⁸

Spectroscopic measurements on fd^{3,4} and Pf1 (R L W and

L A Day, in preparation) show that more than 90% of the residues in the coat protein are in the α -helix conformation. To identify possible non-helical regions, the observed probability of finding residues in α -helical regions (P_α) or β sheet regions (P_β) was used to predict the secondary structure from the sequence⁹. The only two regions of four or more residues with average P_α less than one are indicated by bars in Fig 2. The Pro-6 of fd is included at the N terminus of the α -helical region as observed for several structures⁹. Residues 22–25 of

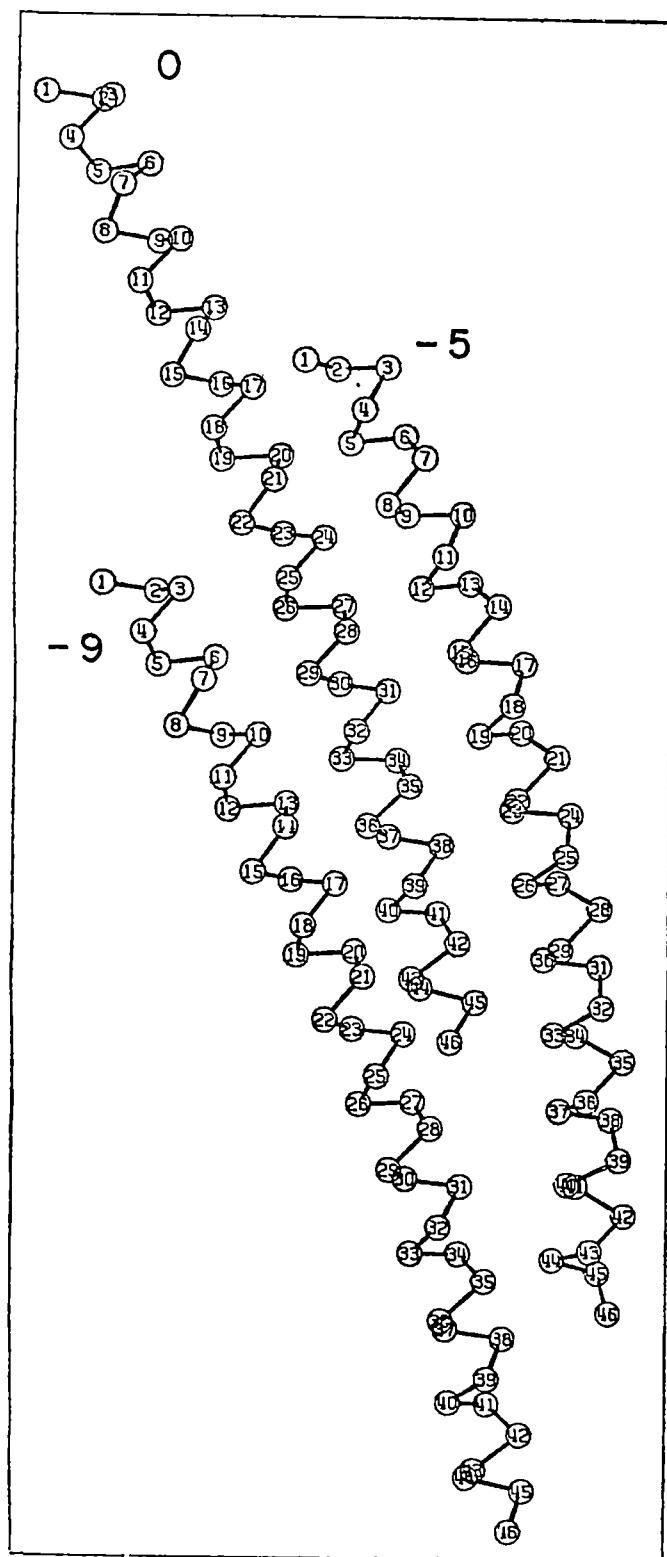


Fig 3 Ball-and-stick representation of α carbon atoms in neighbouring α helices of the current Pf1 model. Atoms are projected on to a plane parallel to the z axis of the virion, to give a view similar to that shown in Fig 5 of ref 10. Numbers indicate residues. The three subunits illustrate the contacts between any subunit (0) and the subunits five units (-5) and nine units (-9) down the virus helix.

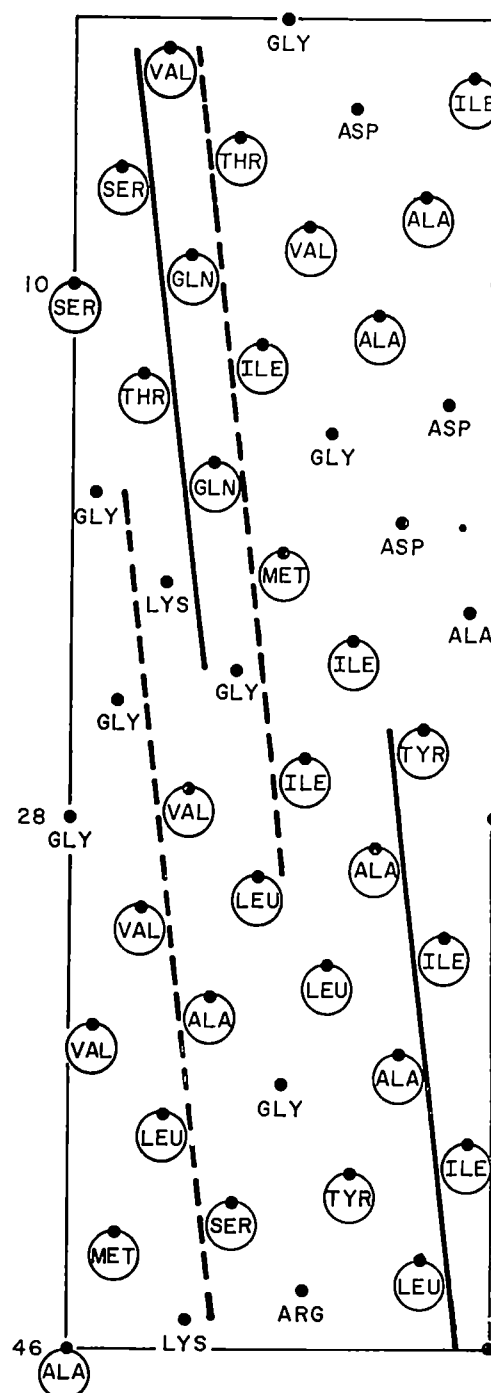


Fig 4 Surface lattice of the Pf1 α -helical coat protein. Positions of residues are projected on to a cylinder which is opened flat and viewed from the outside. Hydrophobic residues or residues involved in van der Waals' contacts are circled (the classification of glutamine, serine and tyrosine as hydrophobic is discussed in ref 14). Dashed lines indicate the lines of contact between the 0 and -5 molecules, and solid lines indicate the lines of contact between the 0 and -9 molecules. In each case the lower line represents the line of contact on the 0 molecule, and the upper line represents the contacts on the other molecule (see also Fig 3).

Table 1 Side-chain interactions in the Pf1 model

Helices 0 to -5				Helices 0 to -9			
Residues	Position*	Type†	Distance (Å)†	Residues	Position*	Type†	Distance (Å)†
17-2	I	—	6.8G	25-2	II	●	>7.0
20-5	II	—	6.4	29-6	I	—	4.5
24-9	I	—	6.0G	32-9	II	●	>7.0
27-12	II	●	5.9	36-13	I	●	4.3
31-16	I	●	5.1	39-16	II	●	6.4
34-19	II	●	5.7	43-20	I	○	4.4
38-23	I	—	4.6G	46-23	II	—	4.8G
41-26	II	●	5.7				
45-30	I	○	4.4				

* Position I and position II refer to the different kinds of sidechain contact on the left and right of the line of contact, as defined in ref 14. A residue in position I is 3 residues on the N-terminal side and 4 residues on the C-terminal side of a residue in position II.

† ●, Van der Waals' contact between β - and γ - or δ -carbons and hydrogens, not involving charged residues. ○, Van der Waals' contact between β - and γ - or δ -carbons and hydrogens, involving charged residues. —, no β - to γ - or δ -carbon and hydrogen van der Waals' contact. Taken from Figs 8 and 9 of ref 14.

† Distance between β -carbons. Where one of the residues involved in the contact is glycine (indicated by G), the distance is given to the position that would be occupied by β -carbon if the residue were alanine.

Pf1 (21-24 of fd) could represent a bend in an α helix involving breakage of a few hydrogen bonds, rather than a drastic disruption of the helix. The non-helical region at the N terminus is sufficient to account for the observed fraction of non-helical residues. The N terminus is probably at the outside surface of the virion¹⁰. This non-helical region is reminiscent of the globular head group at the N terminus¹¹ of the α -helical myosin molecule, situated at the outside surface of myosin filaments in muscle.

For study of sidechain interactions, the Pf1 coat protein can be represented as a 46-residue rod of α helix. The placement of the coat protein rods in the virion¹⁰ shows that there are two main types of interaction between proteins from each protein to the proteins originating 5 and 9 units down the ~ 15 Å virus helix (Fig. 3). The axes of neighbouring α helices make an angle of 9-12° with each other over most of the 40 Å interaction region¹⁰. Analysis of knobs-into-holes interaction of sidechains on infinitely long idealised α helices shows that the sidechains of one helix can fit into the space between sidechains on its neighbour only if the two helices wind around one another with an angle of 20° between the helix axes¹². The region of interaction between Pf1 coat proteins, however, is short enough to permit knobs-into-holes packing over the whole of the interaction region in spite of the small crossing angle.

The remarkable resistance¹³ of filamentous bacterial viruses to extremes of pH suggests that the α helices are held together by hydrophobic bonds rather than salt bridges or hydrogen bonds. The details of sidechain interactions were therefore examined using the principles of van der Waals' interaction developed for tropomyosin coiled-coils¹⁴. Sidechain interactions were explored for several rotations of the α -helix subunit about its own axis (all subunits were assumed to have the same orientation). One orientation was clearly better than all others tried (Fig. 4). Most hydrophobic residues (24 out of 32) are directly involved in interactions with residues on neighbouring α helices (this number includes Val-35 and Met-42, residues that are involved in the shorter interaction region between the 0 and the -14 subunits). Not only the general hydrophobicity of the residues but also the detailed stereochemistry of the interactions is acceptable with this orientation (Table 1). Ten out of the sixteen interactions are of the most favourable type¹⁴, involving van der Waals' contact between β - and γ - or δ -carbons and hydrogens, of these only two involve charged sidechains. The remaining six interactions are of a type that would not permit van der Waals' attraction for regularly aligned α helices, but three of these are in the possible non-helical regions (residues 1-6). For two more, the bending of the α helix in the Pf1 model causes β -carbons to approach to within less than 5 Å of each other, so that van der Waals' attraction might even be possible at these positions.

The coat proteins of both class II viruses, Xf¹⁵ and P1, are about 10% smaller than those of the class I viruses fd and If1 (51 residues: Y N, unpublished). The perturbation observed in

the structure of class I but not class II virions might be due to the additional four or five residues at the C-terminal end of the coat protein. The C-terminal ends are presumed to be in the interior of the virus shell¹⁰, so that additional material here could lead to interference and thus to a structural perturbation.

The model-building approach to molecular structure requires that any acceptable model not only gives a calculated Fourier transform sufficiently close to the observed X-ray data, but also makes stereochemical sense at a level beyond the resolution of the X-ray data. The model-building approach has in the past been restricted to structures made of covalently bonded subunits, such as helical polypeptides or polynucleotides. But this approach can also be useful for the filamentous bacterial viruses, even though the subunits are not covalently linked, because of the knowledge available about internal stereochemistry of α helices and about van der Waals' interactions between α helices. The analysis of sidechain interactions presented here indicates that the model proposed for the Pf1 virion is indeed stereochemically acceptable.

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Vaccination of non-human primates against malignant lymphoma

HERPESVIRUSES are the most important candidates for human cancer agents. The Epstein-Barr virus (EBV) in particular is suspected of being the causal agent in Burkitt's lymphoma and nasopharyngeal carcinoma¹. Immunological principles, well understood from vaccine prophylaxis of viral diseases, provide the potential for the eventual control of herpesvirus-induced neoplasia. The development of safe and efficient cancer vaccines with the human candidate herpesviruses is, however, difficult, as there are no reliable *in vitro* or experimental animal methods for detecting oncogenic potential in preparations which might be applicable to the human subject. We are studying the problems of herpesvirus cancer vaccines in non-human primates. Two members of the herpesvirus family, *H saimiri* (HVS) and *H ateles* (HVA), induce malignant lymphoma and leukemia in several New World monkey species^{2,3}. HVS-induced lymphoma in cotton topped (CT) marmoset monkeys (*Saguinus oedipus*) has been used in this study as all monkeys inoculated with HVS regularly develop malignant lymphoma within two months^{4,5}. This investigation describes the effect of a killed herpesvirus cancer vaccine in protecting non-human primates against malignant lymphoma.

in owl monkey kidney (OMK) cell cultures with a constant serum dilution (1/5) and varying tenfold virus dilutions according to standard techniques⁷. Three of the actively immunised monkeys (A, B and C) have been under observation for more than a year (581 days). The serum antibodies appeared 3-4 weeks after the first immunisation and reached their maximal titres 2-3 weeks later. The antibody titres slowly dropped during the first year and rose again after a booster injection (Table 1).

Out of the 42 immunised monkeys 22 were challenged with HVS. The stock virus, grown in OMK cells⁸, was passed through a Millipore membrane filter (450 nm) and serial tenfold dilutions of the filtrate were prepared. Aliquots (1 ml) of each dilution were injected intramuscularly into immunised monkeys and also into non-immunised control monkeys. The infectivity titre (TCID₅₀) of the virus dilutions was determined in parallel in OMK cells.

In the first experiment three immunised monkeys were challenged with the 10⁻³ dilution of the stock virus and three monkeys with the 10⁻⁴ dilution. The TCID₅₀ of the 10⁻³ dilution was 10 and corresponded to 18 LD₅₀ calculated according to the method of Reed and Muench. All of the vaccinated monkeys survived without any signs of disease and have now been under observation for 337 days. All of the non-immunised monkeys which received the 10⁻³ dilution and two out of three control monkeys which were inoculated with the 10⁻⁴ dilution died of malignant lymphoma 34-51 d after inoculation, indicated by gross pathology and histopathology (Table 2). The tumour-bearing animals carried the HVS genome in their peripheral white blood cells as shown by cocultivation with OMK cells and they developed serum antibodies against HVS. In contrast to the tumour-bearing control monkeys, the HVS could not be extracted from fresh peripheral white blood cells of the vaccinated monkeys by cocultivation methods.

Table 1 Development of serum antibodies against HVS in CT marmoset monkeys immunised with killed HVS

Animal A			Animal B			Animal C		
Days after first vaccination	Serum antibodies against HVS		Days after first vaccination	Serum antibodies against HVS		Days after first vaccination	Serum antibodies against HVS	
	NI*	CF†		NI*	CF†		NI*	CF†
14	0.0	c	13	0.0	c	18	1.0	c
27	1.5	c	26	1.7	c	26	2.3	c
40	3.3	1.32	39	3.2	c	42	3.5	1.64
73	3.3	1.16	60	3.2	c	56	3.5	1.64
97	3.3	1.8	83	2.2	c	123	2.5	c
208	3.3	c	153	1.5	c	152	2.2	c
409	2.0	c	222	2.0	c	180	3.5	c
444‡	1.5	c	395	1.2	c	388‡	0.7	c
452	2.8	c	433‡	0.5	c	396	1.8	1.16
			441	3.0	c			

*Neutralisation indices

†Titres of complement fixing antibodies

c, Serum had anticomplementary activity

‡The monkey received a booster injection of killed HVS

The killed vaccine was prepared by inactivation of HVS with heat (56°C for 4 h) and formaldehyde (100 γ ml⁻¹ for 6 days) as previously described⁶. The HVS-specific antigenicity of the killed vaccines was determined by the complement fixation (CF) test according to standard procedures⁷. An HVS-specific antiserum from an owl monkey latently infected with HVS⁸ was used in the CF test. The CF titres of the vaccines ranged between 1/32 and 1/64. Before use, the vaccines were adsorbed on to Aluminiumhydroxydgel (10% v/v of a 3% solution, Behringwerke, Marburg, West Germany) as adjuvant. For immunisation four to six intramuscular inoculations of the vaccine were given to each monkey within 4-10 weeks, starting with 1.0 ml vaccine for the first inoculation and 0.5 ml for each of the subsequent inoculations. The 42 CT marmoset monkeys actively immunised with the vaccine remained clinically well and developed high titres of neutralising and CF antibodies against HVS. The neutralisation tests were performed

In the second challenge experiment a higher dosage of HVS was used. A 10⁻² dilution of the stock virus was given to four vaccinated monkeys and another four vaccinated monkeys received a 10⁻³ dilution. The 10⁻² dilution contained 100 TCID₅₀ which corresponded to 215 LD₅₀. All of the challenged monkeys were resistant to infection with HVS (Table 2) and have now been under observation for 206 days without any indication of tumour development. All of the non-vaccinated controls which received the 10⁻³ dilution and three out of four monkeys which received the 10⁻⁴ dilution died of malignant lymphoma within 36-52 d of inoculation.

In the third experiment the 10⁻² and 10⁻³ dilutions of the stock virus were again used to challenge four vaccinated monkeys in each group. Since the first and second experiment had shown that 1 TCID₅₀ of HVS corresponded to about 2 LD₅₀, the titration of the inocula in non-vaccinated control monkeys was omitted. Both groups of monkeys in this third

Table 2 Challenge experiments 1-3 CT marmoset monkeys vaccinated with killed HVS and non-vaccinated control monkeys were inoculated intramuscularly with serial tenfold dilutions of cell-free HVS

Dilution of HVS stock virus	Non-immunised monkeys			Immunised monkeys			Titre of inoculum LD ₅₀ *TCID ₅₀	
Experiment 1								
10 ⁻³	+	+	+	-	-	-	18	10
10 ⁻⁴	+	+	-	-	-	-	18	1
10 ⁻⁵	-	-	-	not done			≤1	≤1
Experiment 2								
10 ⁻²	not done			-	-	-	215	100
10 ⁻³	+	+	+	-	-	-	21	10
10 ⁻⁴	+	+	-	not done			2	1
10 ⁻⁵	-	-	-	not done			≤1	≤1
Experiment 3								
10 ⁻²	not done			-	-	-	90	
10 ⁻³	not done			-	-	-	9	

+, Monkey died of malignant lymphoma

-, Monkey remained clinically well

*, Determined in OMK cells

experiment which were challenged with 9 or 90 TCID₅₀, corresponding to about 18 or 180 LD₅₀, have now been under observation for 165 d. The monkeys have remained clinically well and do not show any signs of tumour development (Table 2).

These experiments clearly demonstrate that a malignant tumour in non-human primates can be prevented by a vaccine. The neoplasia investigated is a malignant lymphoproliferative disease induced by inoculation of cell-free HVS into CT marmoset monkeys under laboratory conditions. Under natural conditions HVS appears to be transmitted horizontally, similar to other herpesviruses which have been associated with neoplasia. The prophylaxis of the malignancy was achieved with a killed vaccine prepared from the oncogenic HVS by inactivation. The vaccine proved to be safe and efficient as yet. All vaccinated monkeys developed high titres of humoral antibodies against HVS and remained clinically well. The immunity against HVS can be broken, however, by very high challenge dosages, such as 10^{3.8} TCID₅₀ of HVS⁶. Experiments in progress indicate that the vaccinated monkeys are not resistant to malignancy caused by tumour cell transplantation. The tumour prophylaxis with the killed HVS vaccine and the finding that the TCID₅₀ of HVS correlated very well with the LD₅₀ of HVS support the concept that HVS is indeed the aetiological agent of malignant lymphoma in monkeys. The preparation of killed vaccines may be advantageous with respect to the candidate human cancer herpesviruses since it will be very difficult to exclude oncogenicity of live attenuated vaccines before clinical trials. Recently two groups of investigators showed that EBV-induced lymphoid tumours in *S. oedipus*⁹ and in an owl monkey¹⁰. A killed EBV vaccine may be prepared and tested in non-human primates analogous to the HVS cancer vaccine.

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***In vitro* absorption and molecular weight of specific T-cell suppressor factor**

THE lymph node cells of mice rendered unresponsive by the injection of picryl sulphonic acid (PSA) depress the passive transfer of contact sensitivity¹. When the mice are also painted with picryl chloride the lymph node cells produce suppressor factor *in vitro*. This factor depresses the passive transfer of contact sensitivity by immune cells and its production is T-cell dependent². Here we show that suppressor factor(s) has a molecular weight ~50,000 and that it is absorbed by and can be eluted from specific antigen in the form of picrylated albumin attached to Sepharose beads.

Suppressor factor was prepared by injecting CBA mice intravenously with 5 mg neutralised PSA (BDH). The abdomen and forepaws were painted with 0.15 ml 7% picryl chloride (PCI) in ethanol 5 d later. The draining lymph nodes were taken the next day and live cells (1.2×10^7 ml⁻¹) were incubated in Eagle's minimal essential medium (Difco) containing 10% foetal calf serum, penicillin, streptomycin and glutamine for 48 h. The gas phase was O₂ 7%, N₂ 83%, CO₂ 10%. The supernatants were collected by centrifugation (300g, 10 min) followed by top speed on a bench centrifuge for 30 min. Control (inactive) supernatants were prepared from mice which were painted with PCI but had not received PSA.

In the direct test² the fractions were tested for their ability to inhibit the passive transfer of contact sensitivity to PCI by incubating immune cells in them (Table 1). They were also tested in the indirect test² which is based on the fact that normal peritoneal exudate (PE) cells incubated in suppressor supernatant inhibit the passive transfer of contact sensitivity by immune cells. Five day oil-induced (light liquid paraffin)-PE cells were incubated with fractions (1.2×10^7 ml⁻¹, 1 h, 37°C).

Table 1 Absorption and elution of suppressor factor from picrylated and 'oxazolinated' albumin on Sepharose beads

	Increment of ear thickness \pm s.d. at	
	24 h	48 h
Control (inactive) supernatant	4.1 \pm 0.65	5.2 \pm 0.68
Suppressor supernatant	1.8 \pm 0.51	2.3 \pm 0.49
Suppressor factor absorbed with Pic beads*	4.0 \pm 0.62	5.0 \pm 0.78
Suppressor factor absorbed with Ox beads*	2.3 \pm 0.59	2.8 \pm 0.58
Elate from Pic beads	2.2 \pm 0.84	2.9 \pm 0.72
Elate from Ox beads	3.9 \pm 0.47	4.6 \pm 0.50
Control supernatant eluted from Pic beads	3.6 \pm 0.56	4.8 \pm 0.79
Negative control (non-specific swelling)	1.5 \pm 0.54	1.6 \pm 0.30

The supernatants were absorbed three times by picrylated (Pic) or oxazolinated (Ox) Sepharose beads. The supernatants and eluates were assessed in the direct test by incubation with 4 d PCI immune cells (5×10^7 ml $^{-1}$, 1 h, 37°C). They were washed twice and 4×10^7 cells were injected into each of five recipients. The mice were challenged with 1% PCI in olive oil and passive transfer assessed by the increment of ear thickness. This experiment was repeated on two additional occasions with similar results but the Ox-bead absorptions were not undertaken.

* Bovine serum albumin (1%) beads was coupled to activated sepharose 4B beads at pH 6.2 (ref. 3). Picrylation: 0.1% PSA, pH 9.5, 48 h, room temperature. Oxazolonation: 10 ml beads, 20 ml 0.2 M borate buffer pH 8.4, 0.2 and then 0.3 ml, 5% 4-ethoxymethylene-2-phenyloxazolone (BDH) in ethanol, squirted from a syringe, 18 h. The beads were washed with 0.1% Tween, pH 2.3, 0.2 M glycine-HCl buffer, and PBS.

They were spun down, washed twice and mixed with 4 d immune cells and injected into groups of five mice (4×10^7 immune cells, 1.2×10^7 PE cells per recipient).

In a typical experiment, mice injected with immune cells incubated in tissue culture medium (CS_{100%}) give a mean increment of ear thickness of 4.4 units (10^{-3} cm), after challenge with PCI, while mice which received no cells (CS_{0%}) give a mean increment of 1.4 units. The inhibition caused by a fraction (CS_{exp}) is expressed as percentage inhibition:

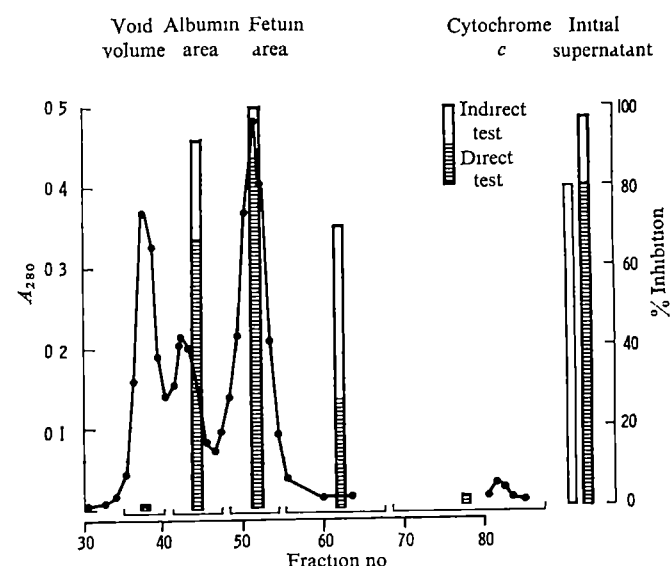
$$(CS_{100\%} - CS_{exp}) / (CS_{100\%} - CS_{0\%}) \times 100$$

Figure 1 shows a Sephadex G-100 experiment. The initial suppressor supernatant gave 97% inhibition. Most of the activity in the direct test (87% inhibition) occurred in the fetus area (molecular weight, 48,000) with less activity in the albumin area (molecular weight 67,000). A little activity eluted after fetus (27% inhibition). These results were confirmed in the indirect test which also showed activity eluting after the fetus area.

Fractions from control supernatant were virtually inactive. The only inhibition found in the direct test was 7% in the

albumin area. In the indirect test, 10% inhibition occurred in the albumin area and 3% in the fetus area.

These results were confirmed in six additional gel filtration experiments. Table 2 shows that in the direct test using fractions from a G-200 or G-100 column there was little or no activity in the void volume and the maximal activity was in the fetus area. With G-50 an average of 77% inhibition occurred in the void volume and a mean of 90% with material which

**Fig. 1** Gel filtration of suppressor supernatant on Sephadex G-100

eluted later. The G-50 data suggest that there may be material with a molecular weight below fetus. This may have been missed in the G-100 experiment because of the loss of material which occurs on freeze-drying from dilute solutions.

The indirect test gave the same general pattern of results but the inhibition in the IgG area was greater. These results were confirmed using Amicon filters PM-100, XM-50 and UM-10. The fractions with presumptive molecular weight above 100,000 and below 10,000 were inactive. Material lying between 100,000 and 50,000 daltons gave 66% inhibition while material between 50,000 and 10,000 daltons gave 89% inhibition.

It was concluded that suppressor factor behaved as though it had a molecular weight close to that of fetus (48,000). It is probably polydisperse. It is not clear whether the material with

Table 2 Suppressor activity of different gel filtration fractions in the direct and indirect test

Sephadex*	Experiment†	% Inhibition of contact sensitivity					Low molecular weight area
		Initial supernatant	IgM area	IgG area	Albumin area	Fetus area	
G-200	1	69(88)‡	6(28)	17(32)	57(53)	80(85)	NA
	2	90	6	23	87§		
G-100	1	69		20	71	83	NA
	2	90(88)		10 (32)	42(97)	84(88)	NA
	3¶	97(82)		0	67(97)	87(100)	27(70)
G-50	1	69				77	89
	2	90(88)				65 (94)	90(100)
Average			6	14(32)	58(82)	75(92)	69(85)

*16 ml supernatant on columns of 1,600 ml, diameter 3.7 cm, eluted with 0.1 M NH₄HCO₃, freeze-dried and reconstituted with 8 ml tissue culture medium shortly before use.

†Experiments with the same number used the same initial supernatant and were tested simultaneously.

‡The number in brackets refers to the indirect test.

§The albumin and fetus areas were combined.

¶Void volume.

||Experiments also shown in Fig. 1.

- a molecular weight in the IgG and IgM area detected in the indirect test is an antibody or antibody-antigen complex or a low molecular weight T-cell product combined with a high molecular weight picrylated protein

The following experiments provide immunochemical evidence for the specificity of suppressor factor and show that it can be specifically absorbed by and eluted from picrylated but not by 'oxazolinated' protein bound to Sepharose beads

Suppressor supernatant (16 ml) was absorbed on to picrylated or oxazolinated beads. For elution the beads used for the first absorption (8 ml) were washed four times in 200 ml ice cold saline and resuspended in 3 ml tissue culture medium with 1% foetal calf serum and heated at 56°C for 30 min. The supernatant was removed and 3 ml of 2 M NaCl added. This was removed after 15 min at room temperature and followed by 0.1 M, pH 2.7 glycylglycine-hydrochloric acid buffer for 15 min. The eluates were dialysed against phosphate buffered saline, pH 7.2, followed by tissue culture medium. Foetal calf serum was added to 5% and the eluate assessed in the direct test.

Table 2 shows that suppressor activity was specifically absorbed by and eluted from picrylated beads. This specific absorption shows that this suppressor factor is not an anti-receptor site factor of the type described by Ramseier⁴. It is not known whether the suppressor factor is a specific T cell product or a complex of the specific T cell product and antigen. In the latter case the molecular weight of the T cell product without antigen is less than about 50,000 daltons and may be much lower.

The suppressor factor described here has a similar molecular weight to the suppressor factors which specifically prevent a tumour rejection (S. Fujimoto, M. Green, and A. H. Sehon, unpublished) and specifically depress the homocytotrophic (presumptive IgE) antibody response⁵. Purification by gel filtration and absorption and elution from antigen opens up the possibility of detailed immunochemical studies.

We wish to thank the Medical Research Council for facilitating this work and the Polish Academy of Science for financial help, and Norton Hadler for technical advice.

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Errata

In the article "Stimulation of synaptosomal dopamine synthesis by veratridine" by R. L. Patrick and J. D. Barchas (*Nature*, **250**, 737, 1974) the following corrections are necessary. Paragraph 2, line 6 should read 'fraction has been shown to be associated with the synaptosomal component' and not as printed. In line 4 of the last paragraph 'interact' should read 'intact'. There were also errors in the Tables which, for the sake of clarity, are reprinted in full below.

Table 1 Effect of tetrodotoxin on the veratridine-induced increase in dopamine synthesis

	Dopamine synthesis (nmol h ⁻¹ g ⁻¹)
Controls	9.54 ± 0.53 (12)
Tetrodotoxin	9.50 ± 0.57 (12)
Veratridine	14.7 ± 0.46 (12)*
Tetrodotoxin + veratridine	10.5 ± 0.43 (12)

Aliquots of the striatal P₂ fraction were incubated for 5 min at 37°C either without further additions or in the presence of tetrodotoxin (2 × 10⁻⁷ M) followed either by the simultaneous addition of veratridine (7.5 × 10⁻⁵ M) plus L-1-¹⁴C-tyrosine (1 × 10⁻⁵ M) or by the addition of tyrosine alone, and incubated for an additional 5 min. The apparent rate of synthesis was calculated by dividing the d.p.m. of product formed per hour per gram of original tissue by the specific activity of the tyrosine added to the medium. The normal incubation medium had the following composition: NaCl, 125 mM; KCl, 5 mM; CaCl₂, 1 mM; MgCl₂, 1 mM; glucose, 10 mM; ascorbic acid, 1 mM (made fresh daily), and Tris HCl, 50 mM, pH 7.4. Values represent the mean ± s.e.m. The number of observations is in parentheses.

*P < 0.001 controls, P < 0.001 tetrodotoxin plus veratridine (t test)

Table 2 Calcium dependence of the veratridine-induced increase in dopamine synthesis

	+ Ca ²⁺	Dopamine synthesis (nmol h ⁻¹ g ⁻¹) - Ca ²⁺ + EGTA	- Ca ²⁺
Controls	13.8 ± 0.22 (18)	13.8 ± 0.41 (18)	12.8 ± 0.72 (10)
Veratridine	18.1 ± 0.30 (18)*	14.4 ± 0.44 (18)	12.0 ± 0.82 (10)

Aliquots of the striatal P₂ fraction were incubated for 5 min at 37°C either in control medium, Ca²⁺-free medium containing 1 mM EGTA, or simply Ca²⁺-free medium, followed by the simultaneous addition of veratridine (7.5 × 10⁻⁵ M) plus L-1-¹⁴C-tyrosine (1 × 10⁻⁵ M) or by the addition of tyrosine alone, and incubated for an additional 5 min. Values represent the mean ± s.e.m. The number of observations is in parentheses.

*P < 0.001 controls

In the article "Dissociation of EEG and behavioural effects of ethanol provides evidence for a noncholinergic basis of intoxication" by W. R. Klemm (*Nature*, **251**, 234, 1974) the following corrections should be made. In the legend to Fig. 1, the dose of eserine (Fig. 1b) should be 0.2 mg kg⁻¹, and of alcohol (Fig. 1c) should be 2 g kg⁻¹. Likewise on page 235, right-hand column, line 27, the dose should be 2 g kg⁻¹.

In the article "Radiocarbon chronology for Seibal, Guatemala" by R. Berger, S. de Atley, R. Protsch and G. R. Willey (*Nature*, **252**, 472, 1974) the first word in line 4, para 1 should read 'stages' and we erroneously gave the address of R. Protsch as Frankfurt am Main, DDR, when in fact it should be West Germany.

reviews

The Social Behaviour of the Bees: A Comparative Study. By Charles D. Michener. Pp. xii+404. (The Belknap Press of Harvard University: Cambridge, Massachusetts, July 1974.) \$25.00.

THE conspicuous social behaviour of bees has undoubtedly been the main reason why they have always stimulated much attention, and continue to do so, with the result that they are kept in the forefront of entomological research. It is indeed a challenging task to attempt to synthesise the accumulated research findings on social bees, and it is one which Professor Michener has ably and brilliantly fulfilled. This book is timely and appropriate and the wealth of recent knowledge gleaned from many sources and many studies is interwoven skilfully around the recurring theme of the origin and evolution of social life to produce a masterly and fascinating exposition. Readers are especially fortunate that Professor Michener's deep and wide knowledge of his subject enables him to put current or fashionable ideas into an objective perspective.

The arrangement of the subject matter of the book into three parts is unusual but effective. Part I is a useful introduction for those unfamiliar with bees. It includes background material about their development, discussion of the features that seem to be involved especially in social behaviour (for example, mouthparts, sting, pollen collecting apparatus and various glands), evolution and classification, and the terminology commonly used.

Bees are unique among social insects in exhibiting a broad spectrum of stages illustrating the evolution of social behaviour. In Part II—the largest part of the book—the types and levels of bee societies and the origins and growth of aggregations and colonies are discussed. Several topics are then taken in turn and for each topic bees exhibiting different stages of social organisation are considered. These topics include the nest and its contents, control of the physical environment within the nest, control of male and female production and sex ratio, caste differences and caste determination, division of labour, colony multiplication, orientation to the nest and food sources, foraging behaviour and communication and colony defence.

I found the chapters on the social



Stephen Dalton

Social organisation of bees

significance of the nest and its contents and on the handling and transfer of materials within the nest to be the most absorbing because from these two chapters one obtains a vivid evolutionary picture of how a series of relatively simple stimuli, and the flow of food and pheromones between the members of a colony, can result in the co-ordinated activities of many thousands of individuals. These subjects have recently undergone intensive research and it is only by a complete and proper understanding of them that we will be able to discover the reasons for population fluctuations and changes in foraging intensity. Such an understanding will, incidentally, help us to use the honeybee more effectively both as a producer of honey and as a pollinator of crops. For example, Professor Michener's initial discovery that the activity of each halictine bee diminishes with an increase in the size of the colony concerned has been extended to other social bees, including the honeybee, for which it has important practical applications. Readers will, however, be fascinated equally by the chapters on caste determination—a subject that is currently attracting much attention—and on the dance language of bees, the interpretation of which has recently been the subject of a controversy that is summarised skilfully and objectively.

The last chapter in Part II, which is devoted exclusively to the origin and evolution of social behaviour, is in many ways the hub of the book. There, Professor Michener discusses the prerequisites necessary for a species to evolve a social behaviour and the

characteristics associated with different levels of social organisation. He also considers how selection could operate to establish various social attributes paying particular attention to kin selection and the role of altruism.

Part III entitled "Natural History" is an account of the locations, architecture and contents of nests, and a discussion of the general biology, life history, behaviour and social status of each of the main groups of social bees, including the semi-social and eusocial halictine bees, euglossine bees, allodopine bees, bumblebees and the stingless and true honeybees. Professor Michener recommends that readers seeking information on a particular group of social bees should read the relevant section of Part III before checking the index for material in Part I and II. Because of the efficient index this system works well in practice, but Part III could, in effect, have preceded Part II. The book is illustrated liberally; the diagrammatic sketches of nest structure and architecture are especially worthy of attention. There is a glossary, and a useful appendix aimed at relating the up-to-date nomenclature used in the book to any older nomenclature the reader may encounter. The book is documented throughout: half of the 700 or so references were published during the past decade and over 80% during the past two decades, reflecting the current interest and progress in the subject.

The entomologist who studies social insects may well find that other insects are somewhat dull by comparison. Readers of Professor Michener's book will appreciate why. **John B. Free**

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Viruses

Comprehensive Virology. Edited by Heinz Fraenkel-Conrat and Robert R. Wagner. Pp. x+191. (Plenum Press: New York and London, 1974.) \$15.00.

VOLUME 1 of this series is unquestionably comprehensive. The *Descriptive Catalogue of Viruses* lists nearly 500 animal viruses, from "Acado" to "Zika", some 350 plant viruses from "Amapari virus (renavirus)" to three viruses of protists from "A" to "ZS", (both bacteriophages of *Escherichia coli*). The descriptions range from purely taxonomic designations — "Amapari virus (arenavirus)" to three pages of detail on tobacco mosaic virus. There are a few errors, a few eccentricities, (an extra 'i' in -viridae throughout), a few omissions (such as the ephemeral fever virus), and perhaps a few mycoplasmas still masquerading as plant viruses. This is, nevertheless, a unique compilation. **J. S. Porterfield**

Choosing and chancing

Our Future Inheritance: Choice or Chance? A Study by A British Association Working Party. Pp. ix+141+4 plates. (Oxford University: London, October 1974.) £4.00 boards; £1.25 paper.

It has become axiomatic that science, the acquisition of knowledge, should be expected to increase the health, wealth and happiness of mankind. That would seem to apply especially to the results of studies on the various complex processes concerning reproduction, whether they be directed at stimulating fertility or curbing prolificacy.

The British Association and its panel of experts are to be congratulated on this publication. The book, though recognising the benefits accruing from advances in biomedical techniques in the field of reproduction and genetics, urges, quite rightly, that a close watch be kept on possible dangers that could arise from their application to disorders in man.

The terms AID, fertility drug, organ-transplant, test-tube baby, and genetic engineering have all become quite commonplace as a result of popular reporting in the media. The methods and results of the scientific procedures associated with these terms are presented accurately. More important is the awareness that is created of the grave consequences that could follow man's deliberate manipulation and possible interference with his own genes, gametes or gonads. The germ cells, which, strictly speaking, are not vital to the individual are well known for their greater sensitivity to environ-

mental and other changes than are somatic cells.

It is intended that the book should be widely read and it deserves to reach all classes of professional and lay people so that the significance of advances in biological sciences, and their application to human medicine, can be fully realised.

The manner of presentation of a scientific subject in a form acceptable to the lay public is problematical but the authors/editors have overcome skilfully a very difficult situation. I get the impression that the book has been presented in such a way that if members of the public who are scientifically uninformed wish to read and understand it they would obtain all that was needed from the first chapter ('Bio-medical advances: a mixed blessing?') and the last chapter ('Social concern and biological advances'). It is regrettable that those two chapters cannot be made more freely available for all to read and enjoy. The intervening chapters contain details about various technical advances and will require a fair understanding of the relevant subjects if they are to have any lasting effect and impression.

The case-bound volume is well produced and contains a useful index.

I. W. Rowlands

Exercise and sport

Science and Medicine of Exercise and Sport. Edited by Warren R. Johnson and E. R. Buskirk. Pp. 486. (Harper and Row: New York and London, 1973.) £10.00.

THIS volume is a collection of 33 chapters compiled by 40 different authors, linked by a common interest in the physiological and medical aspects of sport and exercise. Each contribution is a short review article; the subjects dealt with range from basic biochemical features of contracting muscle to the effects of exercise on the mental state of psychiatric patients. At the end of each chapter is a list of cited references. There is, however, no general index for the whole book.

I found it difficult to determine for whom the text was intended. The general non-scientific reader would find it incomprehensible. A scientist with no specialised knowledge of the subject would find the format difficult reading: it is fragmented, it lacks a theme, and it is therefore indigestible. Perhaps it would be of interest and use to a student writing a dissertation on the scientific and medical aspects of exercise and sport. Its place would therefore seem to be more appropriate to the shelves of a reference library than to a private bookcase.

R. Y. Calne

Rhythm book never swings

Biological Clocks in Marine Organisms: The Control of Physiological and Behavioural Tidal Rhythms. By J. D. Palmer. Pp. x+173. (Wiley: New York and London, June 1974.) £6.85.

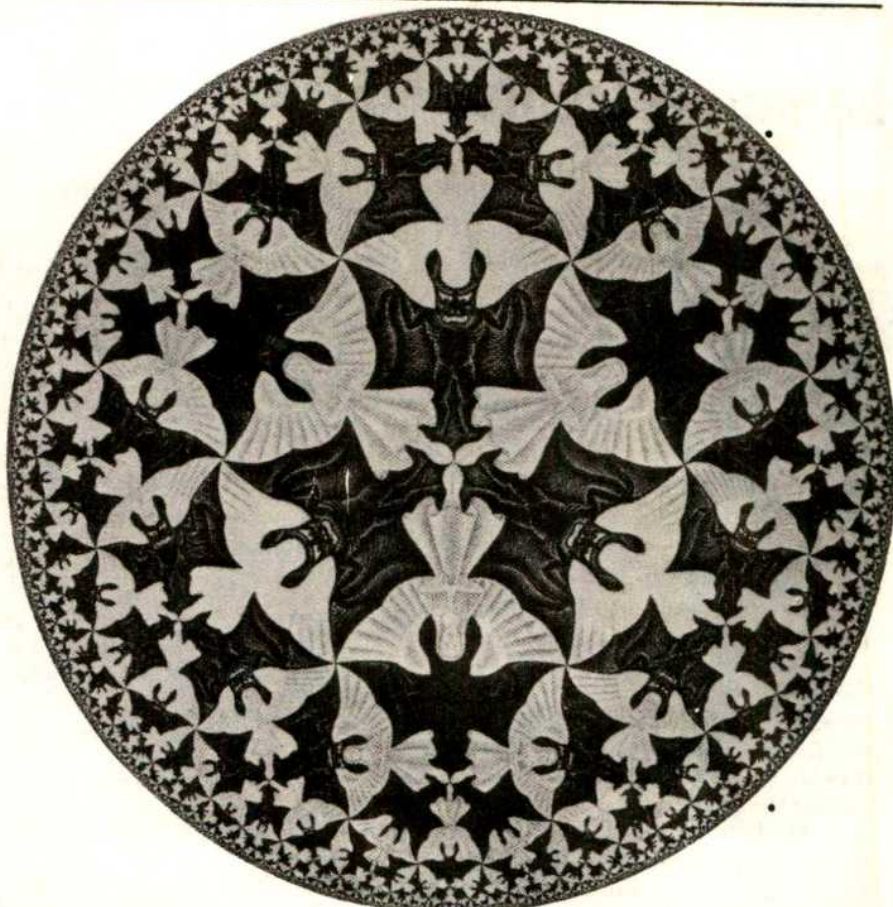
In this book the author attempts to bring the problems and fascinations of the study of biological clocks in marine organisms before a wide audience. The book is, in fact, an expansion of the author's recent review in the journal *Biological Reviews* and is the first of its kind.

It is about the way in which various activities of a whole range of marine animals are instigated and controlled by some facet of the rhythm of the tides. An essential aim of any animal is to maximise the efficiency of, for example, its feeding so that it takes the greatest advantage of food when available. Thus, an intertidal animal which is quiescent when the tide is out undergoes changes when the tide is due to cover it so that it is absolutely ready to feed when water covers it. This preparatory behaviour persists for a period when the animal is removed to a laboratory, demonstrating its innate character. The description of a variety of phenomena of this kind, and the mechanisms behind them, form the substance of this book.

To be completely successful in attracting a wide audience requires subject matter of considerable fascination, a style of writing which is both easily comprehensible and stimulating, and illustrations of high quality. In order to attract future researchers the gaps in present knowledge require emphasis, as do promising lines of research. Unfortunately, although some of these ideals are approached quite closely in some respects the book seems to fall short of its target.

The book is quite comprehensive and incorporates almost all of the significant literature on the subject. It is very well illustrated and the style is generally clear once technical terms are mastered. A very good feature is the summary at the end of each chapter, and the bibliography is of value to the non-specialist.

In spite of that and the obvious expertise of the author, however, I was not greatly stimulated by his treatment of the available material, which seemed rather mundane. I feel that although the general reader is likely to be attracted by the subject he will not be altogether attracted by its presentation; and whether any potential researcher would be fired with enthusiasm is debatable. Dr Palmer does point out the gaps in current knowledge but he never makes one feel that life would



"Circle Limit IV (Heaven and Hell)", a woodcut by Maurits C. Escher. Devils and angels alternate repeatedly and compete for attention. From *Image, Object, and Illusion*. (Readings from *Scientific American*.) Introductions by Richard Held. Pp. 137+155 illustrations. (Freeman: San Francisco and London, 1974.) Cloth, \$8.00; paper, \$4.50.

be incomplete without further knowledge of this or that phenomenon.

The book is undoubtedly a very useful contribution to literature and should be in every biology department's library but its contribution lies in its value as a storehouse of information rather than as a source of inspiration. Its price will probably mean that few other than libraries will buy it.

R. H. Emson

Pain

Recent Advances on Pain. Edited by John J. Bonica, P. Procacci, and C. A. Pagni. Pp. xv+373. (Charles C. Thomas: Springfield, 1974.) \$19.75.

THIS excellent book contains the proceedings of a symposium on the pathophysiology and clinical aspects of pain held in Florence in April 1972. The contributors were eminent men, representing various specialties, from Italy, Switzerland, North America and the United Kingdom.

The main message of the symposium is reflected by its broad coverage—namely, that for both the study of pain and its clinical management a multidisciplinary approach is needed. The subject matter included the neurophysiology of pain, endogenous pain-producing substances, analgesic blocks

and surgery for the relief of pain, pain threshold measurements in man, and operant conditioning for chronic pain. In addition, there is a paper by Professor Bonica on the organisation and function of a pain clinic.

This list, although giving some idea of the scope of this book, does not do justice to the vast amount of information it contains. For those interested in migraine there is a fascinating contribution by Professor Sicuteri, and there are some thoughts on the roles of acupuncture, radiostimulators and inhibitory mechanisms in the relief of pain.

In order that the fruits of many years of research undertaken by the contributors be disseminated as widely as possible this book should be read not only by those whose research is directed towards the study of pain but also by those who are in any way connected with the relief of suffering.

The editing of a book of this kind must be a vast undertaking; none the less, it is a pity that there are so many errors. Apart from spelling mistakes and what seem to be mistakes arising from misread handwriting, it is strange that there are 65 references at the end of Chapter VIII when only nine are mentioned in the text.

Diana R. Haslam

announcements

Awards

The **Royal Society** medals for 1974 have been awarded to the following:

The Copley Medal to **Sir William Hodge**.

The Rumford Medal to **Sir Alan Cottrell**.

A Royal Medal to **Sir Fred Hoyle**.

A Royal Medal to **S. Brenner**.

A Royal Medal to **Sir George Edwards**.

The Davy Medal to **J. Baddiley**.

The Darwin Medal to **P. M. Sheppard**.

The Hughes Medal to **P. H. Fowler**.

The Mullard Medal to **F. B. Mercer**.

The Esso Medal to **K. A. Bray**.

International meetings

February 11–14, **Spectrum Utilisation in Radio Communication**, London (Conference Department, Institution of Electrical Engineers, Savoy Place, London WC2R 0BL).

February 12–14, **Solid State Circuits**, Philadelphia (The Institute of Electrical and Electronics Engineers Technical Activities Board, 345 East 47th Street, New York, NY 10017, USA).

February 21, **Particle Detectors and Dosimeters**, London (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1 8QX).

March 12–14, **Particle Accelerator Conference**, Washington (The Institute of Electrical and Electronics Engineers Technical Activities Board, 345 East 47th Street, New York, New York 10017, USA).

March 17–20, **Conference on Surfaces**, Warwick (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1 8QX).

March 17–25, **Oceanology International 75**, Brighton (BPS Exhibitions Ltd., 4 Seaford Court, 220–222 Great Portland Street, London W1N 5HH).

March 23–29, **5th International Congress on Lymphology**, Buenos Aires/Rio de Janeiro (Professor C. R. Mayall, CP 1822 (2C-00) Rio de Janeiro-GB, Brazil or Dr C. M. Grandval, Austria 2636, Buenos Aires, Argentina).

March 24–25, **Zmuda Memorial Conference on Geomagnetic Field Models**, Colorado Springs (American Geophysical Union, 1707 L Street, NW, Washington, DC 20036, USA).

March 24–26, **Europhysics Conference on Nuclear Interactions at Medium and Low Energies**, Harwell (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1X 8QX).

March 31–April 4, **2nd International Conference on Clustering Phenomena**, College Park, USA (Dr Harry D. Holmgren, Division de Physique Nucleaire, Institut de Physique Nucleaire, BP no. 1 F-91, Orsay, France).

Reports and publications

Great Britain

Department of Industry. Technology and the Environment—Reports from Scientific Counsellors, No. 7. Pp. 56. (London: Department of Industry, 1974.) [1510]

Ministry of Agriculture, Fisheries and Food. Report of the Director of Marine Fishery Research, January 1972/March 1973. Pp. 72. (Lowestoft, Suffolk: Fisheries Laboratory, 1974.) [2110]

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of Central America with Descriptions of Two New Species (Pisces: Characidae). By William L. Fink and Stanley H. Weitzman. Pp. iii + 46. \$1.05. (Washington, DC: Smithsonian Institution Press, 1974. For sale by US Government Printing Office.) [1710]

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January 10, 1975

Few jobs, but plenty of job security

THE graph on our cover this week encapsulates perhaps the greatest problem confronting British universities at present—that of replacing staff.

For every year since 1960 we looked at the classified advertisement section of *Nature* for the first issue in May and the first issue in November. Any British university post of the level of lecturer or above scored one; temporary posts at the lecturer level (there were very few) scored half. The vertical axis therefore represents, roughly, a measure of the long term openings in science in British universities. The graph could be criticised, no doubt, in all sorts of ways as an inadequate sample but the message is clear. Universities have largely stopped hiring, or 1974 contained a statistical fluctuation, or *Nature* has been abandoned as a medium for advertising university posts (British universities generally advertise in two different places). The last proposition was easy to dispatch: nowhere else is there a boom in university advertisements. Statistical fluctuations were ruled out by looking at a larger sample for 1974. So there just aren't the jobs available.

The peak in the mid-1960s, the other salient feature, arose, of course, from the rapid university expansion at that time; indeed in doing a numerical analysis we frequently had difficulty in deciding how many posts were being offered when a university announced that 'several positions are available'!

The policy that university administrations have adopted in the past year, during which severe financial stringency has dictated a widespread freeze in hirings, has been to treat

individual posts which have fallen vacant on their respective merits, and some have said that a year or two of straitened circumstances might positively do good. This is untrue for two reasons. First, when a system with such a high job security ceases to operate normally it works to the detriment of the bright young man trying to move from contract-based employment to a more stable career. Disruption of his job prospects during the most intellectually fertile period of his life represents a tragic waste of education and talent. Second, just as bad news on employment in the chemical industry a few years ago rapidly had an impact on the image of chemistry in schools, it cannot be long before the failure to fill university posts begins to impress students at all levels with a belief that the academic life is being downgraded in national priorities, and that they should not set their sights in that direction. Even if this were a good thing, it is most undesirable that the control should come from external influences rather than from genuine policy decisions made after rational discussion.

It is unlikely that the brakes will be released from universities in the near future; the government doubtless has many other demands on its money which it perceives as more deserving than the universities. In that case, now is the time for universities to respond to the crisis in an imaginative way. Industry, confronted with a constant budget, would find ways of persuading senior or unproductive staff to leave—and it is often possible to do this without any great loss of human dignity. Tenure is both a blessing and a curse to the university system; might not the golden handshake alleviate some of the curse?

Unsuitable for children

PROFESSOR Eric Laithwaite did not, as was widely predicted, quite urge his audience of schoolchildren at the Royal Institution Christmas Lectures (and the several hundred thousand television viewers) to regard Newton's laws as open to some fairly fundamental doubts. He did not say that he had found a way of counteracting gravity. What he did, however, in the course of his six lectures, was in a way more damaging to the education of young scientists; he mixed parlour tricks with complicated machines, he chose to enlist the mystified children on his side in a battle with conventional scientists—'abominable no-men' as he called some of them—and, worst of all, he emphasised experimentation to the almost complete exclusion of explanation.

The scene was as it ever had been—a lecture hall full of well groomed children on their best behaviour. One wondered idly if there were still nannies, governesses and footmen waiting outside in carriages. The audience was prepared to applaud dutifully the most trivial thing. And the experiments—well they can never have been bettered for ingenuity and visual impact.

But science demands rational explanations. It is simply no good demonstrating at length the celebrated mirror problem (if right becomes left, why doesn't top become bottom?) without going to equal pains to put in children's minds at least some sort of answer. It is no good demonstrating a motor that reverses direction when the voltage

is increased without saying why. And it is positively harmful to venture into the world of gyroscopes, a confusing world for many a competent scientist, without a clear understanding of the rather well known laws of angular motion, an extensive reading of the not inconsiderable nineteenth century literature on gyroscopes and tops and an ability to impart the textbook explanation lucidly. The theory is indeed dry and unattractive; all the more challenge, then, to get it right and still hold your audience. Professor Laithwaite chose, instead, to present the understanding of gyroscopic motion as something which had been languishing for more than a hundred years and which scientists as a whole had chosen both to ignore and to suppress questions on.

One example of the misleadingness of demonstration must suffice. A gyroscope slowly precessing on an 'Eiffel tower' seems to experience no centrifugal force, said Laithwaite, otherwise surely the tower would slide, which it didn't do even when the base was on ice, a surface with a very low coefficient of friction. But not low enough. The centrifugal term is sufficiently much smaller than the normal reaction from the weight of the gyroscope (perhaps one hundredth) that sliding requires an even more slippery surface than ice.

These lectures, said Professor Laithwaite, were not the time nor the place to go into the mathematics; but if not, it was neither the time to launch ideas which could only be criticised by mathematical analysis. □

Between combine harvester and ribosome

An Agricultural Research Council/Medical Research Council committee has recently produced a report on food and nutrition research. John Rivers of the Nuffield Institute of Comparative Medicine, London examines its significance.

PERIODICALLY, action by government committees and individuals in this country has resulted in the science of nutrition being rescued from its characteristic doldrums and becoming a national priority. A government inquiry into the physique of recruits for the Boer War got the subject moving in the first place, and later action by people like Lord Boyd-Orr and Sir Jack Drummond gave a new impetus to its growth. These efforts were successful not least because of the military importance of a healthy population. Since 1945, nutrition in the UK has been neither of military importance nor Nobel Prize ranking. It has lost much Research Council support and the standard of the subject and its workers has declined.

The report* of the committee under Professor A. Neuberger is the latest attempt to enumerate nutritional priorities and halt that decline. It examines not only the nutritional status of Britain, but the state of our nutritional knowledge and recommends guidelines for the development of the subject until the end of the century. The strategy adopted is praiseworthy. Priority, it is argued, must be given to "areas of special importance or urgency". Three such are identified—the maintenance and safety of our food supply, the role of nutrition in diseases of complex or uncertain origin, and the extension of our knowledge of the metabolism of nutrients.

Professor Neuberger's committee were asked to prepare a report for both the Agricultural Research Council (ARC) and the Medical Research Council (MRC), an event which is in itself a milestone. The secretaries of the councils welcome it "as a basis for discussion both within the councils and in the wider scientific community preparatory to the consideration of policy". So far the wider scientific community seems, publicly at least, to have been somewhat muted in its discussion. There is therefore the real

possibility that the Neuberger report could, without challenge, become the policy document. This would be a pity, because, despite all its outstanding qualities, the report is a markedly flawed document. The committee has managed to outline graphically some of the priorities of nutrition, but they have remained oblivious of others. The net result is a report which could set back the overall subject by 25 years.

The bulk of the report is a detailed summary of the present state of knowledge in nutrition. Since something over a gross of experts have been consulted, this makes breathtaking reading. If it were expanded and in parts rewritten it would become a necessary primer in nutrition but, as it is now, its function is obscure. It is too condensed to serve as a text book, it is unreferenced and so of little use as a source book, and it is too high powered to be of use as a guide to civil servants working for the research councils or to trustees of foundations. If it is merely there to impress, it succeeds. Moreover it does so in a way that eloquently illustrates Professor Neuberger's conviction that "the answers to many of the most difficult problems in nutrition are ultimately to be found at the molecular or cellular level of biological organisation".

Sir Harold Himsworth—formerly Secretary of the MRC—held a comparable opinion, and some nutritionists felt then that this was a sadly mistaken and reactionary view. Probably they are even sadder now that Professor Neuberger has revived it. Both Himsworth and Neuberger may have described what the MRC choose to fund, but they are not discussing nutrition. In fact they are describing just what nutrition is not. The subject falls somewhere between the combine harvester and the ribosome, but it is not agricultural engineering nor is it molecular biology. It may draw from both but is identical with neither, and it is to be hoped that research councils are aware of its limits when considering which "nutrition projects" to support.

Human nutrition is about the interaction of Man, men and food. It is both a social and a biological science, the domain of generalists who may have some special expertise but remain generalists. Those answers which can only be found at the molecular level, or indeed the economic level, are answers to problems in molecular biology or economics. Both are im-

portant and both should receive funding, but not with money designated for nutrition research.

The apparent erudition of the Neuberger report tempts one, however, to accept the committee's view. The complexity of its science makes it difficult to argue with its insight. Jehovah's Witnesses quote the Bible for much the same effect, but fortunately Professor Neuberger's sources are neither as infallible nor as all-embracing. The result is a review which is eclectic, not exhaustive. Much of what it says is exciting but what it omits is terrifying. Seventy-five pages, for example, are devoted to physiology and biochemistry but only one to ways of influencing food consumption patterns. Even the stress given to different metabolic problems is difficult to understand, unless the length of the contribution is proportional to the status of the author. So although protein and energy metabolism are elegantly viewed from every angle, and coordinated proposals for research in metabolic aspects of obesity advanced, subjects like sucrose and essential fatty acids (EFA) are hardly mentioned.

Yet sucrose provides 15% of the energy of a UK diet—which is a higher fraction than protein supplies. This is a level of consumption which alarms many nutritionists and can delight nobody except the British Sugar Bureau. For some reason the nutrition establishment in the UK seems to regard neither sucrose nor EFA as respectable areas of nutritional research. Perhaps that is the reason why the 27 lines that are devoted to EFA and prostaglandins are so poor. They are marred by misleading omissions, by an erroneous summary of the evidence relating to human requirements and the idiosyncratic phrase "membranes of structural lipids". But the committee's incorrect assertions that "all prostaglandins are metabolically derived from arachidonic acid" or that " γ -lindenic acid is the immediate precursor of arachidonic acid" make the review not only superficial but worthless.

Similar problems occur elsewhere, so that the overall impression is of a report biased in its summary of the literature. Every scientific review is, of course, similarly biased, but since this one gives no references it is impossible to tell whether the author or the reader is ignorant of the literature. Would an unbiased reader really

**Food and Nutrition Research*, (HMSO £3.80).

conclude that studies in high doses of vitamin C have resulted in "very little sound evidence" that it can have any beneficial effect and some reason to suspect it may be harmful"? Or would he remain open minded?

But the scientific review is only the sprat. The mackerel is the set of policy proposals found, in the main, as terse sentences interspersed with the reviews, and designed to give practical expression to Neuberger's three priorities.

Not one of these can be objected to; they are all worth investigation. But then, given enough time and money, so is Uri Geller. If the proposals are considered as the expression of priorities in nutrition many must be rejected. They are the thoughts of experts enthusing over past triumphs and the importance of what they wish to do next. Such enthusiasm is of importance in science, but its expression in this report is sufficiently uncontrolled that a fusillade of ideas have scattered like buckshot around the target. Although Professor Neuberger has sometimes given due priority to an outstanding research need—for example the whole subject of protein and energy metabolism—he has done a disservice by failing to regard any aspect of metabolism as less than a priority.

The committee's approach to the use of animal models also seems to lack coordination. Such models are lacking for many of the intractable problems of nutrition, and the common laboratory species are perversely disinclined to suffer from obesity, diabetes mellitus, kwashiorkor, ischaemic heart disease and many of the other great nutritional scourges. Which is probably why the diseases remain; if the guinea pig possessed L-gulonolactone oxidase, scurvy might pose quite formidable research problems. Primates are proposed at a number of points as a useful model and the limited usefulness of other animals mentioned.

In their views on primates the committee holds an Aristotelian view of animal relationships as a *scala naturae*. This is a view that has been criticised elsewhere with the admonition to choose the model for its particular functional similarity to man rather than on the basis of some vague evolutionary notion of affinity.

There is a case to be made for giving priority to comparative nutrition if we are ever to understand human nutritional disease. Interspecific generalisations like the relationship between energy expenditure and body weight can only improve our perspective on man. When the similarities are established even the species differences can be valuable. If, as the committee suggest, a considerable proportion of basal metabolic rate is accounted for by the energy cost of protein turnover,

how does the cat have a normal energy requirement but a protein requirement twice the interspecific mean?

But above all a comparative approach might teach us to look at animals, their diets and their diseases as an ecological whole. Nutritional epizootology could do much to counteract the myopia of nutritional epidemiology.



Would it work now?

Myopic, too, is the only way to describe the neglect shown by the Neuberger committee of the whole subject of social nutrition. Precious little value is placed on studies of what people eat—and virtually none on the important field of why they eat it, and why they do not. How do we persuade a person to eat what we regard as good, and avoid what we regard as harmful? No amount of heavyweight basic science in nutrition can avoid the fact that food that is not eaten has no nutritional value. Nor can any amount of enthusiasm about action at the molecular level avoid the responsibility of the nutritionists to improve human diets. That responsibility is all too often not discharged because neither nutritionists or physicians know how to alter effectively what people eat.

The omission of any discussion on social nutrition is a curiously blinkered attitude. If it was an error it will no doubt be as unfortunate as it is inexcusable. What young research worker of 'potential' will be attracted now into social nutrition? The subject, anyway, is discouraging—the experiments are often equivocal and research funds are scarce. Presumably funds will now dry up altogether.

It is a pity that Neuberger did not see fit to review the classic work of Barker, Barnicott, Burnett, Douglas,

Joy and Yudkin, or any of the other authors who seem to be aware that food is something more than the sum of its nutrients. If consulted, their views on priorities would have been something more than a lame note on how "it would be of considerable interest to review the results of advertising campaigns . . . and to assess the effect of these on the nutritional value of the diet". We do not know with any precision why or how the UK diet is changing. We do not know why nutrition education fails when advertising works.

Nutritionists are not in business to pursue uncluttered academic research but to improve the nutritional status of the population. Yet they don't know how except by draconian war-time rationing. Meanwhile the pragmatic, arcane arts of the advertising industry are being neither studied nor tested by the academic world.

In the developing world, our knowledge is even more limited. Although nutrition education has proved ineffective as a method of achieving dietary change in the UK it still flourishes as the panacea for Africa's ills. Only recently has there been any serious attempts to see if it works and why it fails. Even now the analyses are oversimplified—for example, no serious attempts are made to identify socio-economic behaviour. Africans are lumped together, or at best split into tribes and nations.

No less urgent is our need to study the sociology of nutritional science. Some field nutritionists have felt that, despite their best efforts, their role as an alien expert inevitably has an overture of racial arrogance. At its worst this is a caricature: the western bourgeois explaining to an African lady how she should prepare one of her own traditional recipes. But even at its least offensive it remains—western man and his science in judgement on another culture and its beliefs. Western man cannot even claim always to have been correct. The life and death of the protein myth, and the distorting effect it has had not only on aid but on the overall development of nutritional science, needs not merely comment but a detailed critical analysis. If we wish to change nutrition, we must understand its structure.

There are two views about the 'golden age' of nutrition. Many scientists feel that this was the vitamin era before the war, when nutrition and biochemistry were synonymous. But to the public it was of most use during the Second World War, when the state of nutrition of the nation improved. It is with use that one must be concerned, and if practical nutrition were given as high a priority as basic science, its 'golden age' could yet be to come. □

international news

USA ratifies chemical warfare protocol

by Colin Norman, Washington

AFTER nearly fifty years of wrangling, the United States government last month belatedly ratified the most important chemical warfare treaty ever negotiated—the 1925 Geneva Protocol, which outlaws the first use in war of chemical weapons. And, for good measure, final approval was also given to a 1972 treaty which forbids the development, production and stockpiling of biological weapons.

But, encouraging as those actions are, they unfortunately do not signify that the Pentagon has lost interest in chemical weapons, nor do they indicate that the Administration is yet prepared to negotiate seriously for international chemical disarmament. Any lingering doubts on that score were dispelled late in December when it became known that even as the Senate was about to approve the Geneva Protocol, the Army was blithely pushing ahead with its plans to produce a new generation of nerve gas weapons called binaries.

For years, the Army Chemical Corps has been conducting research and development on binaries with a view to replacing its ageing stockpiles of nerve agents—estimated to amount to about 40 million pounds—with the new weapons. The idea is that since binaries consists of two relatively non-toxic components which form a lethal agent only when they are mixed together, they can be manufactured and stored much more safely than conventional nerve gases.

But last year Congress refused to grant any money for the production of binary weapons, chiefly because of arguments that if the United States were to embark on a massive new chemical weapons programme, any chances of negotiating an international chemical disarmament (as opposed to no-first-use) treaty would almost certainly have been wrecked. Such negotiations have been taking place in Geneva for the past five years under the auspices of the Conference of the Committee on Disarmament (CCD): it would clearly be difficult for the United States representatives at the talks to retain any credibility if they were placed in the position of arguing for chemical restraint while the Army is busily building up its stockpiles.

Nevertheless, in spite of production ban, the Army published a solicitation in the *Commerce Business Daily* on

December 11, inquiring whether any company would be interested in manufacturing "tor quantities" of two chemical components of binary weapons and also "artillery projectiles filled with a non-toxic chemical solution". That move clearly indicates that the Army has not dropped its plans for binary weapons and that it will almost certainly be coming back to Congress this year with another request for production funds.

Another aspect of the solicitation is also very interesting. Last year, the Army was planning to produce binary weapons with two chemicals which would form the nerve gas GB when mixed together. But the announcement on December 11 indicates that the Army is now also planning to produce a binary weapon which would form the much more persistent nerve agent VX. The gas GB quickly breaks down in the environment, but VX can remain lethal for several weeks under some conditions.

So the Army is clearly hoping to push ahead with its chemical weapons programme, but it will have a tough domestic fight on its hands. For one thing, the arguments which persuaded Congress to shut off production funds last year will be just as strong this year, added to which the new Congress is expected to be politically more liberal than the last. And for another, the

THE announcement last month that the Ford Administration has come round to the view that the Geneva Protocol includes herbicides and riot control agents places the British government in an isolated position. In February 1970 Mr Michael Stewart, then Foreign Secretary, announced in the House of Commons that the British government considers that "CS and other such gases" are outside the scope of the protocol. Although the protocol clearly says nothing about non-military use of chemical weapons, the reason for Mr Stewart's decision was believed to be the fact that CS was being used extensively in Ulster. Even at the time, such an interpretation was shared by few other countries.

● THE British Government was further embarrassed over its policy on nerve gases this week when a *Sunday Times* report revealed that the formula for the lethal VX gas had been

taken off the secret list, and was available to anyone who cared to spend an afternoon browsing in the Patent Office.

The Minister of State for Defence, Mr. Rodgers has agreed to examine the procedures followed when a chemical weapon is taken off the secret list. He gave the undertaking in a letter to Mr. Bruce Douglas-Mann, a Labour MP who tabled two questions to the Defence Secretary Mr. Mason, and expressed fears that the V-agent would be a dangerous weapon if it fell into the hands of terrorists.

Anybody can now obtain a detailed account of the process by which VX is made by applying to the Patent Office, where details were filed 14 years ago as a classified specification. Part of the patent papers were actually reproduced in the *Sunday Times* in order to indicate their availability, and the report suggested that a student, with a little ingenuity, could manage to manufacture the gas in a university labora-

tory. A photograph of the manufacturing apparatus, published after an 'open day' at the chemical warfare plant at Nancekuke, Cornwall, makes the job of the terrorist chemist that much easier.

The nerve gas codenamed VX is the most toxic of a family of V-agents produced by British chemical warfare scientists since World War Two. The gases are organic-phosphorous compounds, and were synthesised in the mid-1950s by a team at the Chemical Defence Experimental Establishment at Porton Down, Wiltshire. V-agents were not produced in bulk in the UK, but under an information-exchange agreement between Britain and America, details of the process were passed on, and formed the basis of a weapons system developed by the US Department of Defence. VX is said to be so toxic that in liquid form a drop the size of a pinhead, placed on the skin, is lethal.

Administration has been carrying out its own internal review of the binary programme. The matter is now said to be awaiting the attention of Secretary of State Henry Kissinger, and it is no secret that the Arms Control and Disarmament Agency (ACDA) has been vigorously opposing production of the weapons. It would clearly be in the Administration's political interest to abort the programme itself rather than face another hopeless battle with Congress.

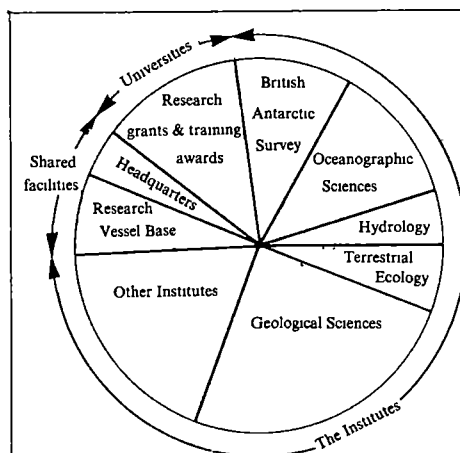
Moreover, even in military terms, the Pentagon may come to realise that the programme just is not worth the candle. It has been estimated that it will cost at least \$1,000 million to replace existing nerve gas stockpiles with binaries and since the Pentagon is always complaining about the inadequacy of the defence budget, it should be asking itself whether the money could better be applied elsewhere.

But, the ratification last month of the Geneva Protocol may at least help the CCD talks when they resume in Geneva this spring. The fact that the United States had not even ratified the 50-year-old treaty barring first use of chemical weapons was viewed by several delegations in Geneva—particularly the Soviet delegation—as evidence that the United States was not really interested in chemical disarmament, but at least that stumbling block has now been removed.

The problems with the protocol came in two parts when it was originally submitted to the Senate for ratification. (All such treaties require a two-thirds majority vote in the Senate before they become official US policy.) First, it fell foul of the chemical industry and was never brought to a vote, after languishing in the Senate Foreign Relations Committee for years, it was eventually withdrawn from the Senate. Then, in 1971, President Nixon resubmitted the treaty to the Senate for ratification.

But another impasse developed because Nixon insisted that herbicides and riot control agents are not covered by the protocol an interpretation which would have meant that, even if the United States ratified the treaty, it could continue using herbicides and tear gases in Vietnam. Senator J. W. Fulbright, Chairman of the Senate Foreign Relations Committee, did not, however, accept that interpretation. He refused to bring the measure to a vote and asked the Administration to review its position.

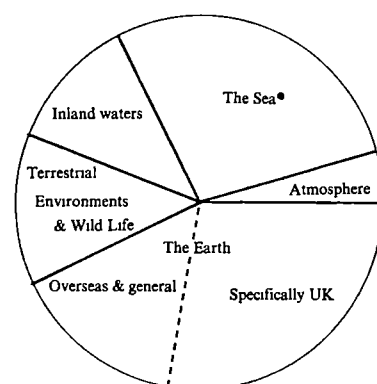
There the matter rested for three and a half years until Fulbright called a meeting of the Foreign Relations Committee on December 10 last year to review the situation. Faced with another embarrassing blaze of publicity the Administration finally overruled the



THE British Natural Environment Research Council (NERC) saw through half of the total Rothschild transfers in the year 1973-74 according to its recently published report (HMSO, £1.50). For that year £3.6 million worth of research was assigned to customer departments, for the most part satisfactorily—meaning that everyone carries on much as before including the NERC which goes on managing. The NERC does, however, sound a warning signal. The council doesn't reckon it has enough applied research under its wing to meet the Rothschild targets, so some "quite basic research" is being transferred to departments. The question is whether in lean times ahead basic research in a customer department's budget will present a "conflict of priorities", a delicate term for being axed.

Some of the transferred programmes do not sit too easily in customer departments. The NERC gives two examples. The geological survey of the continental shelf is a hard-to-place baby. There is £1.5 million of applied research to be transferred, but the first home for it, the Chemical and Minerals Board of the Department of Trade and Industry, turned out to be less than ideal. It looks as if it can find a foster-home with the Shipping and Marine Technology Board of the

NERC's £19 million cake divided—by means and by ends



same department but the NERC would like to see it finally adopted by the Department of Energy. On the other hand, the land Geological Survey is a much sought-after property and in order to prevent it from being torn limb from limb, the NERC has agreed to the survey being overseen by a consortium comprising, *inter alia*, the NERC's chairman, Sir Peter Kent, and representatives of the departments of Trade, Industry, Environment, Scottish Development and of the Welsh office.

The Institute of Geological Sciences is reported to be finding difficulty in recruiting and keeping staff to work on the independent analysis of North Sea commercial data that it attempts to carry out. Uncompetitive salaries in the scientific civil service are blamed.

Further, significantly fewer people applied for research studentships this year, the report notes, but those that did were distinctly more willing to move to a different university for their second degree.

As the figure shows, universities continue to comprise only a modest fraction of the NERC's commitment. Britain's terrestrial toe-hold in the Antarctic, gets almost as much as the total expenditure in universities.

Pentagon, and Dr Fred Iklé, Director of ACDA, announced that President Ford now accepts that the protocol bars first use in war of all chemical weapons, including herbicides and tear gases. The measure then sailed through the Senate without opposition.

The actual effect of all this on progress in the CCD talks is uncertain at present, largely because of the looming threat of the binary programme. But, assuming that talk of producing binaries dies away this year, the chief stumbling block in Geneva will become the difficulty of making sure that a chemical disarmament treaty can not

be violated. One possibility, however, is that a treaty banning further production of lethal chemical weapons will first be negotiated. That would prevent chemical proliferation—and also reduce the capability of both the United States and the Soviet Union, since existing nerve gas stocks will slowly deteriorate. A ban on stockpiling would then be negotiated later. A Japanese proposal along those lines is now receiving close attention in the United States.

The key to the whole business, however, is what happens to the binary programme. □

WHILE international attention has been focused on plans for nuclear power stations in Israel (and neighbouring countries), Israeli scientists have continued their search for new sources of energy, or for more efficient means of exploiting old sources. Everything is being considered, from the proposal of a scientist at the Haifa Technion to construct 10,000 giant energy-generating windmills to the scheme of his colleague for a thermonuclear power reactor.

Research continues on an obvious energy source in this sub-tropical climate—the Sun. Unfortunately, solar energy has not proved itself economically viable except for the heating of household water supplies. And its benefits in this sphere are cancelled out, in the eyes of environmentalists, by the ugliness of the roof-top solar energy installations which, together with the omnipresent television aerials, manage to create a particularly horrifying urban skyline.

Even though Israel has far more ample supplies of solar radiation than of water, there is even talk of constructing a 120-MW hydroelectric station near the northern end of the Sea of Galilee.

A small hydroelectric station was actually opened on the River Jordan, south of the Sea of Galilee, way back in 1932, but it has been out of action since the 1948 war. The proposed new plant would be placed at a point where

the Jordan plunges 830 feet down into the northern end of the Sea of Galilee.

Utilisation of oil shale is also under active consideration thanks to Professor Yehoshua Schachter of Bar-Ilan University, an Orthodox Jewish institution where all the scientists wear skullcaps. Professor Schachter believes that the estimated 700 million tons of oil shale recently discovered in the Negev Desert would be sufficient to generate some

Letter from Israel

from Nechemia Meyers, Tel Aviv

1,000 MW of electricity for 30 years. This is an impressive figure in Israeli terms, as the country's present generating capacity is 1,600 MW.

Schachter's plan, now being studied by a government-appointed engineering company, calls for the shale to be burnt on-site to generate electricity, rather than for the oil to be extracted first and then burnt at a power station. Such a direct burning process has been used successfully in West Germany and Estonia, and skyrocketing fuel prices have made it look attractive here.

Professor Schachter also emphasises the fact that the power-generating facility he suggests would be particularly valuable in wartime, when other fuel imports might be cut off.

• War, of course, could come here at almost any moment, and in the mean-

time Israel has a full-scale anti-terror campaign of her hands, one in which scientists have already played some role. Their contributions, unfortunately, have not always been utilised. For example, a reliable Israeli-developed device for 'sniffing' explosives was not used to check the explosives-laden suitcase of a young man who arrived recently at Lydda (Ben-Gurion) Airport. The next day he used the explosives to make bombs which he subsequently exploded inside a Tel Aviv cinema.

• Israel's special problems made it the natural venue for an International Conference on Psychological Stress and Adjustment in Time of War and Peace, which opened at the University of Tel Aviv on January 6. One of the papers presented at the conference described a study carried out by Dr Avner Ziv, University of Tel Aviv, on 'shelter children', youngsters in border areas who must spend extended periods in underground shelters because of recurrent shellings. Dr Ziv and his colleagues found that kibbutz children under bombardment adjusted to the stresses of the situation much more successfully than did children at nearby small towns. He speculates that the highly organised social structure of a kibbutz, and the fact that it operates almost like an extended family, may provide a more secure atmosphere for children in times of crisis than does the nuclear family in a town.

AFTER a year and a half of actively, but silently consulting everybody, the Dutch Science Minister Mr F. H. P. Trip, finally ventured into the limelight to present his 'Purple Book' on science policy.

Mr Trip is a most remarkable man. He has roots in industry (as a managing director of Naarden Chemie, a specialised company with more people in its laboratories than in its production departments) and in academe (as President-elect of Utrecht State University), so many regarded him as the ideal science manager. Also, surprisingly, he is a member of one of the most radical political parties in the Netherlands.

Yet, according to his new plans, he is not after radical changes in the structure of science policy. The universities maintain their rate of income per student and their freedom to handle the sum of Dfl 1,000 million as they please. But they are requested, if not required, to set up a reasonable book-keeping system for their research projects, in accordance with the government's technical guidelines. In view of the present state of the average university administration, implementation of this rule will call for a major effort.

Mr Trip's purple book

from Arie de Kool, Rotterdam

Something like Dfl 100 million a year is now handed out by the ZWO, the organisation for pure scientific research. It is rightly being claimed that this rather insignificant amount has a very considerable steering effect. The Foundation for Basic Medical Research funded by the ZWO, claims to coordinate about Dfl 80 million worth of research on a budget of Dfl 4 million. They get there by adding a man here and a centrifuge there—on conditions, of course. The ZWO (and the Royal Academy, as far as its own institutes are concerned) will be transformed into a Council for Scientific Research (RWO), with departments for fields like physics, life sciences and social sciences. Each department could have many 'working groups', consisting basically of everybody working in a specific discipline. There could be working groups for solid state physics, molecular biology, sociology of the family and so on.

The working groups elect ten mem-

bers of the department, five others are appointed by the Academic Council (the cooperative institution of the universities), and five more, together with a president, by the government.

Some Dfl 1,000 million is spent on more or less applied research sponsored by the government. This will stay under direct ministerial control although the ministers concerned will receive advice, and even planning schemes to cover several years from sectorial councils, such as a council for environmental problems, one for maritime research or the environmental movement.

The councils are to be meeting points for government and producers and consumers of science. So each council consists of some government representatives, some scientists from the institutes concerned and some people from 'society'—industrialists, labour union representatives and people from the environmental movement.

Mr Trip also hopes that industry (in the Netherlands some 75% of industrial research and development is done by five multinational companies) will be open about its own projects, so that good coordination of government-funded research and development will be possible. □

correspondence

UNESCO

SIR,—As visiting scientists at the Weizmann Institute, we find the recent UNESCO anti-Israel resolution doubly abhorrent

First, as scientists and educators, we are appalled at the biased, politicised and cowardly vote, which has made a travesty of an organisation established to help wipe out just such ignorance and prejudice

Second, being in Israel we have had the opportunity to explore Jerusalem—the old, the new, the restored, the excavated—and thus to recognise the absurdity of the claim that the excavations carried out in the Holy City have changed its character

We call on our fellow scientists to join us in protesting UNESCO's moral bankruptcy and in demanding that the organisation abandon politics and return to dealing with science, education and culture

Yours faithfully,

L ANDERSON (University of Illinois), E E A BROMBERG (University of Wisconsin), C BRUNK (University of California), D CAHEN (Northwestern University), A COGOLI (ETH, Zurich), S COHEN (State University College at Buffalo), R COOPER (University of Pennsylvania), B S DUDOCK (State University of New York at Stony Brook), S EDELSTEIN (Cornell University), D FAIMAN (CERN), A FRIMER (Harvard University), R A GELMAN (Case Western Reserve University), A KRSTOSEK (University of Oregon), S J LEBOVICH (University of Washington), J MANZ (Technische Universität, Munich), S B MIZEL (Colorado State University), J PRIVES (Columbia University), H SCHMITT (Free University of Brussels), J SCHULTZ (University of California), P B SIGLER (University of Chicago), A SOUDAK (University of British Columbia), M THALER (University of California), S WEISROSE (University of London), H YAMASAKI (Hiroshima University), M ZEICHER (Vrije Universiteit, Belgium)

Another African Chalicothere

SIR,—Among a large collection of vertebrate fossils from the Lukeino Formation which I made recently I found the proximal phalanx of a large Chalicothere. Its size suggests that it belongs to *Ancylotherium hennigi* Dietrich, although if so that species will prove to be the oldest known specimen (~6.5 Myr)

Chalicotheres are now known from several deposits in Africa—the Lower Miocene of Rusinga and Songhor, Kenya, the Pliocene of Kaiso, Uganda, from which the first African Chalicothere was recognised (C W Andrews, *Nature*, 112, 696, 1923), and Lower Pleistocene deposits such as Olduvai, Tanzania (P M Butler, *Bull Br Mus Nat Hist Geol*, 10, 165–237, 1965). In the Baringo area Kenya, they have been recognised from the Chemeron Formation (~4 Myr) and now from the Lukeino Formation



On discovery of the Lukeino fossil, I described a Chalicothere to my field assistant, Mr Kiptalam Chepboi, who assured me that I had accurately described a *Chemosit*. I repeated the description to several other local people, all of whom gave the same opinion. The *Chemosit* is an animal of Kalenjin myth, on which the 'Nandi Bear' is supposed to have been based (*Nature*, 112, 696, 1923 and B Heuvelmans, *On the Track of Unknown Animals*, Rupert Hart-Davis, London, 1958) and it was the first discovery of a Chalicothere in Africa that prompted Andrews to enquire whether the Chalicothere was still extant and whether it formed the basis of the myth. It is therefore of great interest to obtain further information from an independent source, separated by more than two generations and hundreds of miles, that indeed the *Chemosit* and the Chalicothere closely resemble one another

It is unlikely that the Chalicothere is still extant, even in the depths of the Zaire forests, but it is not inconceivable that it survived until the recent past, entering local mythology before finally dying out

MARTIN PICKFORD
Queen Mary College, London

Asbestosis

SIR,—W P Howard's comment (December 13) on Peter J Smith's article (October 18), "For those in peril on the factory floor" which dealt with the industrial hazards of asbestos, itself deserves comment. It is true that conditions in the industry are incomparably better than they were in some factories 40 years ago but the occurrence of an average of 139 new cases of asbestosis a year in Britain still emphasises a formidable hazard. Mr Howard, writing from a London office where he only inhales the few asbestos fibres normally present in an urban atmosphere, may have every confidence that in coming years the number of cases will be reduced to a very low level indeed, but this confidence suggests a complacency that is not, I am sure, characteristic of the industry as a whole. We do not know to what level the concentration of asbestos in the atmosphere must be reduced before the hazard will disappear. The Maximum Admissible Concentration level is a guess

That asbestos is a dangerous commodity cannot be too strongly emphasised and, although it is indispensable for certain purposes, it should be used with discretion. Because of television programmes describing the occurrence of asbestosis and cancer in workers in Hebden Bridge, the public has become aware of dangers that can arise when the material is handled indiscriminately. A general review (*The Biological Effects of Asbestos*, distributed by the World Health Organisation) that summarises the report of a working conference on the biological effects of asbestos, sponsored by the International Agency for Research on Cancer, indicates that all the major commercial types of asbestos can cause cancer and that there is strong evidence to relate past exposure to asbestos with mesotheliomas, a distressing cancer of the pleura that covers the lung

The danger may not relate only to employees. One paper in the same report represents a study of the incidence of mesotheliomas in the city of Hamburg-Bergedorf in the years 1958–68. In the city as a whole the incidence was 0.056%, in the residential area around one factory it was 0.96%. Cases considered to be occupational were excluded

P F HOLT
University of Reading

Research in India

It is only natural that Dr Macdonald (*Nature*, November 15, 1974)—with his World Health Organisation (WHO) and Genetic Control of Mosquito Unit (GCMU) connections—should have reacted the way he has, to a part of my item on work on the genetic control of mosquitoes (*Nature*, September 20, 1974) I am afraid I cannot agree that there was anything “misleading”, “misinformed” or “erroneous” in statements which pointed out disparities between population needs and the GCMU’s priorities in choosing its programmes. The military implications too cannot be summarily dismissed, as Dr Macdonald has done, by merely hiding behind the familiar generalisation that all research results, in theory, could be misused. The fact of the matter is that there have been instances of misapplications. It may well be recalled that the defoliants employed by the US Army in Vietnam were reported to have been first tested for their effects in some Latin American countries. I cannot say what were the specific long-term benefits from these tests to the Latin American Communities.

Then there is the case of childhood malnutrition. For years now, we have been led to believe (by all kinds of agencies including UN and others) that protein deficiency in the Indian diet is the major cause of malnutrition in India. Consequently, vast amounts of Indian (as well as foreign) money were diverted towards high-protein food programmes, whereas the problem all along—we are now told—has been one of simply not enough food.

Moreover, studies “specifically designed for the long-term benefit of the community” should surely be able to stand a little scrutiny, and a probe into the whole affair can only result in clearing the air and, I hope, lend support to contentions like Dr Macdonald’s, and I and many others in the scientific community shall be happier for it. Little inconveniences like these, I am afraid, will remain a foreign scientist’s lot (not only in India but elsewhere) so long as instances of the academic and scientific communities providing cover for nefarious activities by their governments continue to surface.

Yours faithfully,
NARENDER K. SEHGAL

Jullundur

SIR,—The belated comments on United States Defense Department and National Museum activities in India by N K Sehgal and A N D Nanavati (*Nature*, September 20, November 29) need to be considered in relation to

some other past history.

So far as I can make out the most revealing information is provided by William E Small in *Scientific Research* (3, (25), 27, December 9, 1968), where investigations of bird diseases transmissible to man in Brazil and the biology of the north central Pacific by the Smithsonian Institution (the US National Museum) were identified as supported by the US Defense Department chemical and biological (CB) warfare research centre at Fort Detrick in Maryland. The resulting furore reached a climax the following February, as reported in the *Washington Post* and *New York Times* on February 5 and the London *Times* and *Guardian* next day, where it is alleged that Baker Island in the central Pacific had been chosen for tests. Eventually President Nixon announced on November 25, 1969 that the USA would abandon CB warfare except for a small defensive programme, though the *Times* reported on September 21, 1971 that stocks of tularaemia, anthrax, Q fever and Venezuela equine encephalitis organisms were retained and work continued at Dugway, Utah, the place where 6,000 sheep were inadvertently killed in a mismanaged nerve gas experiment in March 1968.

Although this activity may suggest that the US Defense Department, in collaboration with the Smithsonian Institution, also financed a Migratory Animal Pathological Survey from Korea through the Far East and India to the eastern Mediterranean at much the same time, as far as one can make out the actual work was normally delegated to irreproachably upright citizens who succeeded in making very good use of the funds provided, to such an extent that the people most likely to take offence, the Russians (who were incidentally carrying out similar activities of their own), were happy to co-operate, as reported by Mr Nanavati, though the Chinese refused to do so. In consequence the withdrawal of US funds following criticism of the Defense Department has resulted in a sad gap in ornithological work in the Far East especially, where one could wish for a less controversial alternative source of money. Mr Sehgal may rest assured that whatever the original object of the Migratory Animal Pathological Survey, a lot of people have scrutinised its activities rather carefully and found nothing to complain about except some roughness in handling captive birds, so that many of us who were once among the foremost critics of its possible original object would now like to see some means found to keep it in being.

As many people have already remarked, it also seems a pity that the

Smithsonian Institution ever allowed its activities to become so closely associated with those of the US Department of Defense, and it hardly seems surprising that people in India remain suspicious.

Yours faithfully,

W R P BOURNE

University of Aberdeen

Ghost families

SIR,—There are even families of ghost writers (December 6) *Proc 5th int Conf Soil Mech* (1961) lists in its Author Index not only F Asce (II, 105) but also his more prolific younger brother M Asce (I, 517 and II, 117). Is there a case for forming a Society of Irreproducible Scientific Authors?

Yours faithfully,

PHILIP I LEWIN

Building Research Station,
Garston, Watford, UK

SIR,—May I add to the Christmas season of ‘ghost authors’ with my own favourite. A paper on a mermaid foetus is quoted in the Cumulative Index Medicus, and also by at least one subsequent author, as Williams, H I, and Lumpur, K, (1962) *Arch Path (Chicago)*, 74, 472. In fact reference to the paper itself shows that Dr Williams, the sole author, wrote his report from Kuala Lumpur.

Yours faithfully,

MARTIN D’A CRAWFURD

The University,
Leeds, UK



A hundred years ago

THE great solar eclipse of 1868 was visible in Siam, as the 1875 eclipse will be. The then reigning Siamese king had not invited any European astronomer, but the French Government sent an expedition, who located themselves in Malacca for the purpose of taking spectroscopic observations. The King of Siam, who professed to be an astronomer, came with a royal train and a large army to observe the sun and perhaps the sun-observers. The observations were very successful indeed, but the French astronomers had located themselves on marshy land and were almost all attacked by fever, of which they were cured only on their return to France. Such was not the case, however, with their royal guest, who was also attacked, and died a few months afterwards from *Nature*, 11, 216, January 14, 1875.

news and views

Understanding the control of vitamin D synthesis or taking a short cut through South America

from a Correspondent

CHOLECALCIFEROL is classified as a vitamin only because its synthesis from 7-dehydrocholesterol is effected by ultraviolet light rather than by an enzyme. Consequently if there is limited exposure to sunlight, the diet must be supplemented with cholecalciferol. 1,25-dihydroxycholecalciferol ($1,25-(OH)_2D_3$), the active form of cholecalciferol, is more properly defined as a hormone since it is synthesised solely in the kidney¹ from its immediate precursor 25-hydroxycholecalciferol ($25-(OH)D_3$). This metabolite is involved in translocation of calcium across cell membranes and in calcium homeostasis, and has potential uses in the treatment of metabolic bone disease. There is therefore much interest in the control of $1,25-(OH)_2D_3$ production and, since the chemical synthesis of $1,25-(OH)_2D_3$ cannot be carried out in high yield, in natural sources of the hormone.

The control of $1,25-(OH)_2D_3$ production has now become a source of controversy with two possible mechanisms being proposed, a recent paper² has indicated the complexity of the relationship of $1,25-(OH)_2D_3$ with parathyroid hormone. Most groups believe that $1,25-(OH)_2D_3$ production is primarily under the control of parathyroid hormone. This is supported by the changes which occur in chick mitochondrial $25-(OH)D_3$ -1-hydroxylase activity in response to changes in parathyroid hormone concentrations³. Also within hours of thyroparathyroidectomy the concentrations of $1,25-(OH)_2D_3$ fall in blood and intestinal mucosa of rats on a low calcium diet, but injection of parathyroid hormone restores $1,25-(OH)_2D_3$ concentrations to normal⁴. The rate of conversion of $25-(OH)D_3$ to $1,25-(OH)_2D_3$ in an isolated renal tubule preparation can be increased by the addition of parathyroid hormone or cyclic AMP to the incubation media⁵. Both the concentrations of $1,25-(OH)_2D_3$ in tissues of rats and chicks and the activity of the $25-(OH)D_3$ -1-hydroxylase enzyme measured *in vitro* are responsive to changes in levels of calcium in the plasma⁴ and media³ respectively. But these groups felt that calcium does not

control $1,25-(OH)_2D_3$ production first, because the enzyme activity measured *in vitro* was not related to the plasma calcium concentrations in the bird from which the kidneys were obtained³, second, the transfer of rats from a low calcium to a high calcium diet results in a rapid decline in plasma calcium concentrations whereas the $25-(OH)D_3$ -1-hydroxylase activity remains elevated for very much longer⁶. Calcium affects the activity of the $25-(OH)D_3$ -1-hydroxylase enzyme by preventing electrons from flowing into the 1-hydroxylase system from a specific NADPH generating system. Under these conditions added NADPH provides the necessary reducing equivalents⁷.

The alternative mechanism for control of $1,25-(OH)_2D_3$ production has been proposed by Professor MacIntyre and his group⁸. They have not seen an increased production *in vivo* of $1,25-(OH)_2D_3$ in response to parathyroid hormone⁹ nor in their isolated renal tubule preparation could parathyroid hormone raise $1,25-(OH)_2D_3$ production though dibutyl cyclic AMP did have a positive effect¹⁰. If calcium was omitted from the incubation medium however, parathyroid hormone did stimulate $1,25-(OH)_2D_3$ production. Since they found added parathyroid hormone to be increasing cyclic AMP concentrations in their isolated renal tubules they have postulated that the influx of calcium into kidney cells, known to occur in response to parathyroid hormone, has negated the effect of the increased cyclic AMP concentrations. They have now incorporated their findings in a hypothesis which proposes that $1,25-(OH)_2D_3$ production is subject to feedback control.

The key observation was the decreased capacity of renal tubules to synthesise $1,25-(OH)_2D_3$ if the tubules had first been pre-incubated for 150 min with the hormone. This inhibitory effect of the $1,25-(OH)_2D_3$ was not due to competition by the hormone for the active sites on the $25-(OH)D_3$ -1-hydroxylase enzyme and was not detected if the renal tubules were incubated with the hormone in the presence of

actinomycin D. The authors propose that $1,25-(OH)_2D_3$ stimulates by a nuclear action the synthesis of a protein, possibly calcium-binding protein, which is involved in the mechanism of calcium uptake by kidney cells. In the presence of this protein, tubular calcium is reabsorbed and the consequent raising of kidney cell calcium concentration inhibits $1,25-(OH)_2D_3$ production. There is therefore a cycle of events in which $1,25-(OH)_2D_3$ production is controlled by its own action on the tubular reabsorption of calcium.

Crucial to the validity of this hypothesis are the limits to any variation in the concentration of cellular calcium. This information is unfortunately difficult to obtain with confidence because under certain conditions mitochondria take up extremely large amounts of calcium. Even so the changes in $25-(OH)D_3$ -1-hydroxylase activity which have been observed in response to changes in calcium concentration are inadequate to explain the changes in $1,25-(OH)_2D_3$ production *in vivo* unless the cell calcium concentration varies over enormous ranges. Thus a 7.5 fold change in calcium concentration gives rise to only a 50% inhibition in $1,25-(OH)_2D_3$ production *in vitro*, whereas several-fold variations in $1,25-(OH)_2D_3$ production rates can be observed *in vivo*³.

These variations might, however, have other causes. For example, any measures that lower serum inorganic phosphorous and hence kidney phosphorous concentrations increase $1,25-(OH)_2D_3$ production⁴. In addition there may be multiple effects of parathyroid hormone on $1,25-(OH)_2D_3$ synthesis. Favus, Walling and Kimberg² have found that the response of rats to parathyroidectomy varies with time. They found that there is a diminished intestinal calcium transport 72 h after surgery, but parathyroidectomised rats adapted to a low calcium intake over 21 d. This adaptation to a low calcium diet is known to be $1,25-(OH)_2D_3$ dependent. In interpreting these and other results it should, however, be appreciated that an absolute dependence of $1,25-(OH)_2D_3$ production on

the presence of parathyroid hormone has never been shown. In parathyroidectomised animals some 25-(OH)₂D₃-1-hydroxylase activity always remains. In accord with this Favus *et al* found plasma concentrations of the hormone were low but levels in the intestine were normal 21 days after parathyroidectomy.

Recently the South American plant *Solanum malacoxylon* has been seen as a possible source of 1,25-(OH)₂D₃. Cattle and other animals eating this shrub show features similar to those of hypervitaminosis D including hypercalcaemia, hyperphosphataemia, vascular and ectopic calcification, wasting and eventual death. The derangement of calcium metabolism probably derives mainly from the increased intestinal absorption of calcium. There is some evidence that the active principle in *S. malacoxylon* is 1,25-(OH)₂D₃ rather than the other vitamin D metabolites¹¹.

S. malacoxylon given orally to chicks induces synthesis of calcium-binding protein (CaBP)^{11,12}. This protein seems to be involved in calcium absorption and its synthesis is dependent upon 1,25-(OH)₂D₃. The action of the leaf is not inhibited by the inclusion of strontium in the diet, a metal which inhibits the conversion of 25-(OH)D₃ to 1,25-(OH)₂D₃ by the kidney enzyme system, suggesting that the active principle is not metabolised by the kidney 25-(OH)D₃-1-hydroxylase prior to its action in the intestine. Corradino and Wasserman¹³ have shown that the aqueous extract of the leaf has two other actions similar to that of

1,25-(OH)₂D₃. Thus it is active in stimulating within 12 h CaBP synthesis and calcium absorption in organ cultured chick duodenum, again implying that if any metabolism of the active principle is involved it can occur in the intestine. As in the case of 1,25-(OH)₂D₃, the induction of CaBP in this system is inhibited by actinomycin D. In addition 1,25-(OH)₂D₃ and the aqueous extract of the plant induce *de novo* CaBP synthesis in 18-day-old chick embryo duodenum, whereas 25-(OH)D₃ is much less potent in the embryo in this regard and vitamin D seems to be without activity.

Little information is available on the chemical nature of the active principle. If it is 1,25-(OH)₂D₃ then its water solubility implies that it is attached to large polar moieties and the hydrolysis of this complex may account for its delayed action^{12,13}. Its identification is awaited with interest.

- ¹ Fraser, D. R., and Kodicek, E., *Nature*, **228**, 764-766 (1970).
- ² Favus, M. J., Walling, M. W., and Kimberg, D. V., *J. clin. Invest.*, **53**, 1139-1148 (1974).
- ³ Fraser, D. R., and Kodicek, E., *Nature new Biol.*, **241**, 163-166 (1973).
- ⁴ DeLuca, H. F., in *Metabolism and Function of Vitamin D* (edit by Fraser, D. R.), 5 (Biochemical Society, London, 1974).
- ⁵ Rasmussen, H., Wong, M., Bikle, D., and Goodman, D. B. P., *J. clin. Invest.*, **51**, 2502-2504, (1972).
- ⁶ Omdahl, J. L., and DeLuca, H. F., *Physiol. Rev.*, **53**, 327-372 (1973).
- ⁷ Ghazarian, J. G., and DeLuca, H. F., *Archs Biochem. Biophys.*, **160**, 63-72 (1974).
- ⁸ Larkins, R. G., MacAuley, S. J., and MacIntyre, I., *Nature*, **252**, 412 (1974).
- ⁹ Galante, L., Colston, K., MacAuley, S., and MacIntyre, I., *Lancet*, **i**, 985-988 (1972).
- ¹⁰ Larkins, R. G., *et al*, *Clin. Sci. molec. Med.*, **46**, 569-582 (1974).
- ¹¹ Wasserman, R. H., Bar, A., Corradino, R. A., Taylor, A. N., and Peterlik, M., in *Proc. Vth Parathyroid Conference* (edit by Talmage, R. V.) (Excerpta Medica Amsterdam, 1975).
- ¹² Lawson, D. E. M., Smith, M. W., and Wilson, P. W., *Febs Lett.*, **45**, 122-125 (1974).
- ¹³ Corradino, R. A., and Wasserman, R. H., *Nature*, **252**, 716 (1974).

dence), has meant that these drugs can be used as pharmacological tools to study the function of endogenous prostaglandin release in the body (see Ferreira and Vane, *The Prostaglandins*, volume 2, edit by Ramwell, Plenum Press, New York, 1974 for review).

An alternative way of studying physiological events in the absence of prostaglandins is to actively immunise animals against them, prostaglandins can easily be made immunogenic by coupling them to a carrier protein such as bovine serum albumin. Active immunisation against prostaglandins has recently been shown to afford a certain protection against inflammatory stimuli (Ferreira *et al*, *Prostaglandins*, in the press). Using similar techniques Horton and Poyser have obtained further evidence that prostaglandin F_{2α} is involved in luteolysis in the guinea pig (*Prostaglandins*, **5**, 349, 1974). This experiment adds further support to the idea (now widely accepted) that prostaglandins are involved in luteal regression in several animal species (Pharris, *Peispec. Biol. Med.*, **13**, 434, 1970) though apparently not in man. Other aspects of the reproductive process are also susceptible to modification by prostaglandins (see Persaud, *The Prostaglandins*, volume 2, edit by Ramwell, Plenum Press, New York, 1974). Prostaglandins are in clinical use for the termination of pregnancy and induction of labour and an interesting corollary to their stimulating action on the myometrium is the manner in which aspirin-like drugs delay parturition in both man and animals (see Persaud, cited above). These effects reinforce the view that endogenous PG production in the uterus is the final pathway for expulsion of the contents.

When Vane made his observation that aspirin inhibited prostaglandin biosynthesis in a cell-free system (*Nature*, **231**, 232, 1971) there was already some evidence indicating that prostaglandins were involved in the pathogenesis of inflammation and fever. Thus, as Vane pointed out, inhibition of prostaglandin biosynthesis could explain the well known anti-inflammatory and antipyretic effects of aspirin. Since then a link has been established between inhibition of prostaglandin biosynthesis and the analgesic actions of aspirin-like drugs (Ferreira, *Nature new Biol.*, **240**, 200, 1972; Ferreira, Moncada and Vane, *Br. J. Pharmac.*, **49**, 86, 1973). Important features of the aspirin-like drugs are that the analgesia they produce is mild and that they act peripherally (Lim, *Arch. int. Pharmacodyn.*, **152**, 25, 1964). By contrast the narcotic analgesics are extremely potent drugs which act within the central nervous system and do not have any direct effect on prostaglandin biosynthesis. Recently,

Aspirin, prostaglandins, endoperoxides and platelets

from R. J. Flower

DURING the first six months of 1974 almost 500 publications on prostaglandins were cited by *Index Medicus*, this compares with some 400 publications *per annum* two years ago, approximately 170 in 1970, fewer than 100 in 1968, and only 25 in 1966. It is not difficult to understand this tremendous interest in prostaglandins: almost all mammalian cells possess the enzymatic machinery (generally known as 'prostaglandin synthetase') for the synthesis of prostaglandins from precursor fatty acids; furthermore, biosynthesis and release of prostaglandins from cells can be evoked by a wide range of physiological, pharmacological or pathological stimuli, and the different prostaglandins which are produced have a wide spectrum of pharmaco-

logical activity in concentrations often as low as 10⁻⁸M. These properties make prostaglandins obvious candidates for mediators of a wide range of physiological events.

Physiological functions

Enormous impetus has been given to research on their physiological function by the discovery that the 'aspirin-like' drugs inhibit prostaglandin biosynthesis (Vane, Ferreira, Moncada and Vane, Smith and Willis, *Nature*, **231**, 1971). These observations have since received widespread confirmation. The blockade of prostaglandin synthesis by drugs of the aspirin type, being unique, specific, and common to almost all tissues and all species so far examined (see Flower, *Pharmac. Rev.*, **26**, 33, 1974 for evi-

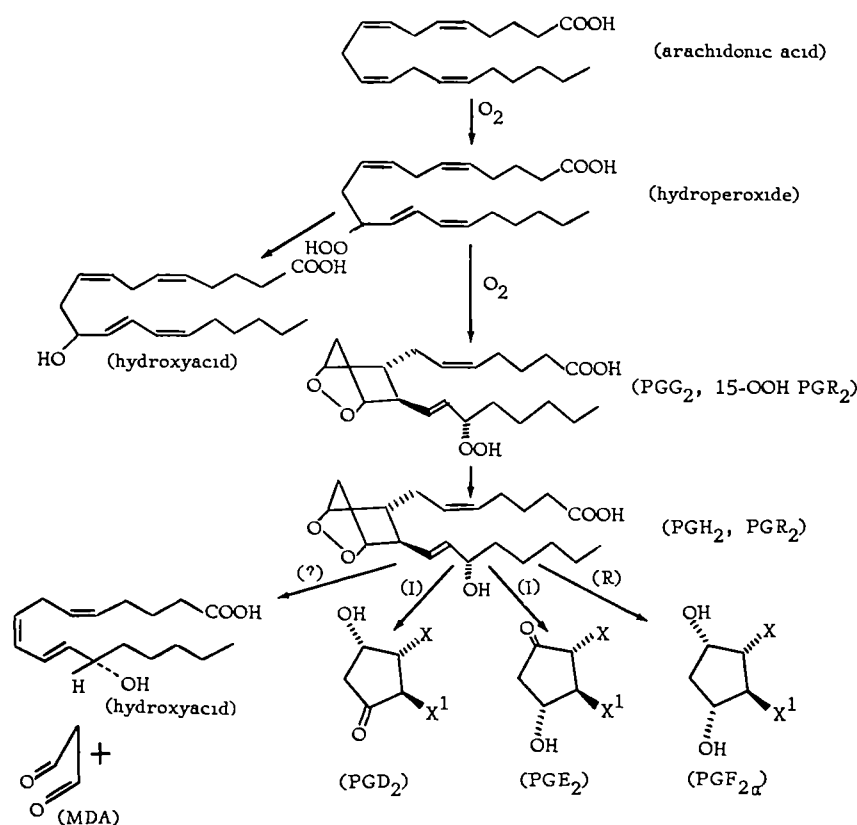


Fig 1 Proposed reactions for generation of cyclic endoperoxides and prostaglandins from arachidonic acid This scheme is based largely on the work of Nugteren *et al*, *Rec Trav Chim Pays-Bas*, **85**, 405, 1966, Nugteren and Hazelhof, *Biochim Biophys Acta*, **326**, 448, 1973, Hamberg and Samuelsson, *J biol Chem*, **242**, 5336, 1972, *Proc natn Acad Sci U S A*, **70**, 899, 1973 and Hamberg *et al*, *Proc natn Acad Sci U S A*, **71**, 345, 1974 Only the ring structures of the prostaglandins have been drawn, X and X¹ refer to the appropriate hydrocarbon chains I=isomerisation, R=reduction, ?=enzymic or non-enzymic MDA=malondaldehyde Although this scheme shows the conversion of arachidonic acid into G₂, H₂, E₂, and so on, the conversion of di-homo- γ -linolenic acid probably proceeds by an analogous pathway

however, an interesting and provocative hypothesis by Collier's group (see *Nature*, **248**, 241, 1974, and **252**, 56, 1974) suggests that these narcotic drugs may exert their effects by preventing the stimulation of brain adenylyl cyclase by E-type prostaglandins

Endoperoxides—the 'intermediates'

One of the most significant advances of the last two years has been the isolation and characterisation of two intermediates in the conversion of precursor fatty acids to prostaglandins. These intermediates are of interest to the biochemist because prostaglandin synthesis is an unusual type of oxygenation reaction. They also interest the pharmacologist, for Gryglewski and Vane (*Br J Pharmac*, **45**, 37, 1972) have suggested that 'rabbit aorta contracting substance' (RCS—a potent smooth muscle contracting factor first reported by Piper and Vane in 1969 whose release from perfused guinea-pig lungs during anaphylaxis is blocked by aspirin) was an intermediate in prostaglandin biosynthesis. Hence many workers had been anxious to test the biological activity of the intermediates.

The precursors of prostaglandins are the 20-C essential fatty acids. There are several groups of prostaglandins distinguished by the nature and geometry of substituent groups in the ring (for example E, F, D) and the number of double bonds in the side chains (for example E₁, E₂, E₃, F₁, F₂, F₃) all of these can be produced by the synthetase complex from the appropriate precursor. In 1965 Samuelsson proposed the existence of a common intermediate—a "cyclic endoperoxide"—which once formed could break down to either E, F, or D type prostaglandins. Two such endoperoxides have now been isolated by Samuelsson's group (*Proc natn Acad Sci U S A*, **70**, 899, 1973, *ibid*, **71**, 345, 1974) and by Nugteren and Hazelhof (*Biochem Biophys Acta*, **326**, 448, 1973). The first 'stable' product to be formed by the oxygenation reaction is called by Samuelsson's group 'prostaglandin (PG) G' and by Nugteren and Hazelhof '15-hydroperoxy PGR', the second intermediate has been named 'PGH' by Samuelsson and 'PGR' by Nugteren and Hazelhof (see Fig. 1). Both the endoperoxide intermediates are very unstable in aqueous solution,

spontaneously decomposing to prostaglandins (half-life approximately 5 min). They can, however, be isolated in some organic solvents and are stable in dry acetone at -20°C for some months.

Their instability in aqueous media has led to difficulties in the testing of the biological activity of these 'prostaglandins'. Generally the endoperoxides have greater smooth muscle contracting activity than the prostaglandins. On the isolated rabbit aortic strip the endoperoxide had a biological activity of 1-2 orders of magnitude greater than those of prostaglandins themselves and initially it seemed as though the activity of the endoperoxides could account for the RCS of Piper and Vane. But a careful comparison of the properties, especially of the breakdown rates of the endoperoxides and of RCS, has made this identity less likely. Thus in addition to the endoperoxides there may be at least one more biologically active intermediate in prostaglandin biosynthesis.

Endoperoxides also seem to have a potent biological action as platelet aggregating agents. In 1973 Willis and Kuhn (*Prostaglandins*, **4**, 127, 1973) reported that preparations of prostaglandin synthetase generated a "labile aggregation-stimulating substance" (LASS). This substance was rapidly generated enzymatically from arachidonic acid (but not from other related fatty acids) and its formation was blocked by aspirin. Early in 1974, a further paper by Willis (*Prostaglandins*, **5**, 1, 1974) described the properties of LASS, observing that it "cannot be distinguished" from the endoperoxides. Almost simultaneously a report by the Swedish group appeared (Hamberg *et al*, *Proc natn Acad Sci USA*, **71**, 345, 1974) in which they reported that purified preparations of PGG₂ or PGH₂ were extremely potent platelet aggregating agents in concentrations of 10–300 ng ml⁻¹, furthermore during aggregation of platelets with thrombin, endoperoxide was released in similar concentrations into the medium. Later in 1974 a similar report appeared (Smith *et al*, *J clin Invest*, **53**, 1468, 1974) confirming and extending these findings. Interestingly, only the endoperoxides from arachidonic acid (that is, the precursors of G₂, E₂, F₂, D₂ and so on) aggregate platelets, the endoperoxides formed from di-homo-γ-linolenic acid (the precursor of G₁, H₁, E₁, F₁, D₁ and so on) do not.

These data help to explain why inhibitors of prostaglandin biosynthesis such as aspirin block platelet aggregation, whereas PGE_2 has only a very weak aggregating action and PGE_1 actually inhibits aggregation. It seems that in platelets the final products of prostaglandin synthetase are relatively inactive end products of the synthetic

reaction and one wonders whether a similar situation obtains in some other systems. Since prostaglandin synthesis (hence endoperoxide generation) can be initiated by damage to cells and tissues, workers from each group have pointed out that endoperoxides are most likely the trigger for platelet thrombus formation at wound sites.

Doses of aspirin which block PG synthetase are (generally speaking) well tolerated, implying that prostaglandin synthetase is not an enzyme vital to the existence of the organism. This accords with the view that prostaglandins are modulators of physiological processes, perhaps mainly involved in local communication between cells, especially in defensive reactions induced by damage or stress. But, because of the exponential increase in prostaglandin literature, a few more years will have to elapse before prostaglandins are assigned a definite role among the body's homeostatic mechanisms.

Ecologists classifying plants

from Peter D Moore

It is said that when a soldier is confronted by a static object he will be inclined to paint it, a biologist placed in the same situation will attempt to classify it. By the beginning of this century, when the vast bulk of larger animals and plants had been placed into seemingly appropriate taxonomic pigeonholes, a rising breed of biologists, now classified as ecologists, began the formidable task of sorting entire communities of species into meaningful groups. Starting with the belts of climatically determined vegetation on a global scale, they worked their way down to the level of habitats. In Britain this descriptive age culminated in the production of Tansley's *The British Islands and their Vegetation* (Cambridge, 1939), in which vegetation types were defined on simple criteria, mainly the dominant species. On the Continent a more elaborate system was developed by Braun-Blanquet in which vegetation types were characterised by plant species which were confined to any given association (so-called 'faithfuls'), these were considered to be of narrower ecological amplitude and therefore more specific indicators of the environment than were the dominants used in the British system. In a modified form, this system remains the standard approach to vegetation description and classification throughout the European mainland.

In Britain the Braun-Blanquet system has never really established itself, partly because of the general poverty

Another rock term bites the dust

from L. F. Penny

At last the enigma of the Hesse Till is solved. This, the topmost member of the Devensian (last glaciation) boulder clays on the east coast of England, which most geologists have hitherto regarded as a till sheet in its own right, is now shown by Dr Madgett to be nothing more than the soil profile at the summit of the succession (this issue of *Nature*, page 105).

To be sure, this solution has occasionally been suggested before. Eighty years ago Carvill Lewis suspected it (*Glacial Geology of Great Britain and Ireland*, 209, 1894). Bisat was getting warm in 1940 when he found that the Hesse Till inland (Madgett's Zone C) was different from that on the coast (Zone A), but he was not thinking of a pedological explanation (*Proc Yorks geol Soc*, 24, 148, 1940), and more recently the same doubt has been expressed by Fenton (*E Yorks Field Studies*, 2, 3, 1969). But suspicion is one thing, and proof is another, it is this that is now presented, as a result of the application of modern pedological methods to what was previously regarded as a purely geological problem. Briefly, the so-called Hesse Till in Yorkshire lies sometimes on the Drab Till and sometimes on the Purple Till, and it is now identified, where it overlies the Drab, as weathered Drab, and where it overlies the Purple Till, as weathered Purple. And this is why Bisat's "inland Hesse" (which lies on Drab or directly on Chalk) was different from the "coastal Hesse" (which lies on Purple).

If we ask why such a neat and satisfactory solution has never been proved before, the answer must be that the wrong people have been looking at it. Madgett is a pedologist, who has brought a pedological

eye to the examination of that part of the succession which geologists usually dismiss in their field note-books as "Soil 2 m". The story of the Hesse Till shows how dangerous it is for geologists to draw conclusions about superficial deposits without taking pedological advice (as indeed it would be for a pedologist to interpret a soil profile without an understanding of the geological history of the area). In several countries it is now normal practice for Pleistocene sections to be examined simultaneously by geologist and pedologist, who write it up together, in Britain we are moving toward this more integrated approach, largely due to the activities of interdisciplinary groups such as the Quaternary Research Association, but it is still true to say that much more could be done (and mistakes avoided) by a greater awareness in each group of what the other can contribute.

Clearly, as Madgett suggests, the term Hesse Till should now be dropped. Hesse has never been a good type locality, and if the till there is really weathered Drab, there is no point in perpetuating the name. Perhaps these discoveries will stimulate a more comprehensive revision of the nomenclature of the east coast tills. Are "Drab" and "Purple" really satisfactory as stratigraphical names? Is "Hunstanton" any better than "Hesse", if there too the till is weathered Drab? Probably the time has come to redefine them according to modern usage, in which each lithostratigraphical division is "designated by a distinctive proper name and defined in a specified place or region" (*Int geol Correlation Programme*, UK Contribution, Royal Society, 1968).

of our flora and partly as a consequence of the penetrating critique directed at it by Poore (*J Ecol*, 43, 226, 1955). Unconvinced about the real existence of plant communities as definable entities, researchers in Britain and in the United States and Australia have preferred to regard vegetation as a continuum of variation in its specific composition. This being so, the most convenient approach to classification has been the erection of reference points within the continuum at appropriate locations (Poore, *J Ecol*, 44, 28, 1956). As an aid in this task, the tech-

nique of ordination has been used extensively, in essence this involves the comparison of stands of vegetation on the basis of their floristics and the construction of geometrical, spatial models which can best account for the various similarities and differences observed (for example, Bray and Curtis, *Ecol Monogr*, 27, 325, 1957).

Hierarchical classification of stands, reminiscent of the Braun-Blanquet type, has found a place in British plant ecology mainly as a result of the work of Williams and Lambert (*J Ecol*, 47, 83, 1959), who initially used a chi-

square statistic for stand comparison and later an information statistic either for splitting or for agglomerating samples into the most homogeneous groupings possible (Williams, Lambert and Lance, *J Ecol*, **54**, 427, 1966, and Lance and Williams, *Computer J*, **11**, 195, 1968). These methods have provided a degree of objectivity which is not usually a feature of Braun Blanquet classifications, but whether they provide more information to those concerned with community descriptions has often been questioned (see Frenkel and Harrison, *J Biogeogr*, **1**, 27, 1974).

In the United States, Whittaker (*Ecol Monogr*, **23**, 41, 1953) provided a new approach to the classification of vegetation. He plotted stands along axes representing environmental factors, such as moisture availability and altitude. By substituting at each point the abundance of a given species in that stand he was able to ordinate species with respect to their ecological requirements and in this way learn something of the species and consequently of the nature of the community. It is something akin to this approach which Grime has recently used (*Nature*, **250**, 26, 1974) in his analysis of grassland communities, but instead of employing environmental factors as axes he has taken physiological and morphological characteristics of the plants themselves. He uses log maximum relative growth rate on one axis and competitive index (defined in terms of morphology and litter production) on a second axis and is thus able to locate any species in relation to these two parameters. He can substitute vegetation stands for species on such axes by using the mean maximum relative growth rate and mean competitive index of the species found within the vegetation sample. Vegetation from different habitats produces clusters of points in various parts of the graph and Grime accounts for these positionings in terms of stress and disturbance factors. In habitats where stress and disturbance are low, species of high competitive index predominate (see Grime's earlier model of species density variation with environmental stress, *Nature*, **242**, 344, 1973). Species of low competitive index are found mainly in stressed and disturbed habitats, those in the former having generally low growth rates and those in the latter having higher ones. Grime's concept of three major determinants in vegetation composition (that is, stress, disturbance and competition) has led him to use a triangular form in his ordinations.

The usefulness of this new approach to the classification of vegetation will depend upon the validity of this tripartite factor model of plant communities in habitats other than grassland. Is it always possible to

discern between these ill-defined categories of stress and disturbance? Is maximum potential growth rate of a species either an adequate measure of normal growth potential or an adequate criterion for the differentiation of stress- and disturbance-adapted species? We must await data from other habitats. Meanwhile it is refreshing to find a system which steps beyond classification for its own sake and seeks to provide a predictive model upon which management and conservation programmes can be based.

Oceanic lithosphere thickens with age

from Peter J. Smith

At active oceanic ridges there is a regional elevation resulting largely from thermal expansion and perhaps in part from phase changes related to thermal state. Some of the uplift here may also be due to an anomalously thick asthenosphere with an abnormally low density near the ridge crest. But outside the immediate vicinity of the spreading axis, where such arguments no longer apply, the case for variation in lithospheric thickness is much less certain. Most theoretical models of seafloor spreading have therefore assumed that the lithosphere moving away from a ridge has a constant thickness.

Experimentally, the range of determined lithospheric thickness is wide, although most values lie between 40 km and 110 km. For example, upper mantle sections derived from surface wave dispersion observations suggest that an oceanic plate is 70 km thick on average, but other estimates of the same quantity range from 40 km to 100 km. For continental regions there are recorded thicknesses up to 100 km in tectonic belts and up to 150 km in stable shields. Using estimates of the flexural rigidity of the lithosphere, Walcott (*J geophys Res*, **75**, 3941, 1970) calculated the thickness of the normal continental lithosphere to be 110 km and that of the oceanic lithosphere to be 75 km or more. Magnetotelluric methods, on the other hand, have given 10 km thickness for the zone of spreading on Iceland and 40 km for the subduction zone below Kamchatka, the latter value having been confirmed by studies of the attenuation of shear waves.

In short, although existing experimental data probably give lithospheric thickness to the correct order of magnitude, they are far from sufficient for testing whether or not the lithosphere produced at any given ridge has a constant thickness. Moreover, as Vogt (*Earth planet Sci Lett*, **23**, 337, 1974) now points out, there is an inherent

danger in comparing directly determined lithospheric thicknesses insofar as different methods do not necessarily define lithospheric plate in the same way. Rheologically, plate thickness is the maximum depth at which brittle fracture can occur; magmatically, it is the minimum depth at which partial melts are ubiquitous; thermodynamically, it could be defined as the pressure-temperature level at which partial melting begins in a system with known or assumed physical properties, and so on. Not only are these definitions not necessarily identical, the lithosphere-asthenosphere boundary may not even be sharp.

For the time being, therefore, direct experimental determinations are of limited use and recourse must still be made to theoretical models. But the problem with theoretical models involving constant lithospheric thickness is that the thickness is assumed rather than predicted by the model itself. In the important model proposed by McKenzie (*J geophys Res*, **72**, 6261, 1967), for example, lithospheric thickness is a free parameter not determined by the physics, and it was to overcome this difficulty that Parker and Oldenburg (*Nature phys Sci*, **242**, 137, 1973) put forward a modification which enabled them to predict that, beyond 10 km from a ridge axis, the thickness of the lithosphere should increase in proportion to the square root of age.

To test which, if either, view is correct—the traditional assumption or the Parker-Oldenburg prediction—Vogt has adopted and adapted a method first used by Eaton and Murata (*Science*, **132**, 925, 1960) to determine the minimum depth from which magma must rise to reach the summit of Mauna Loa volcano, Hawaii. Eaton and Murata assumed that at the depth of the magma source the magma column exerts the same lithostatic pressure as the surrounding normal crust and upper mantle, including water and sediment (that is, there is isostatic equilibrium). The source depth thus determined was 57 km, and although the calculation was carried out before the days of plate tectonics, this depth agrees with the lithospheric thickness beneath Hawaii obtained by more modern means (for example, from the level of deepest seismicity). Vogt therefore believes that the magma is formed beneath the lithosphere and that what Eaton and Murata were measuring was, in effect, the plate thickness.

It is implicit in this that volcanoes such as Mauna Loa cease activity because of an isostatic limitation and not simply because no further magma is available. If this argument may be applied to most volcanoes, it then follows that there should be an observable relationship between volcano

height and plate thickness, in other words, it should be possible in principle to determine plate thickness indirectly from volcano height. In practice, this will only be possible for active or recently extinct volcanoes because older volcanoes will have been eroded and their heights lost for ever. Moreover, there are almost certainly bound to be some volcanoes which cease activity because of insufficient magma and others that have not yet reached their full height, and in these cases heights will also be too low to conform with any volcano height-plate thickness relationship.

But what do the data show? From the mathematics of the isostatic system, Vogt concludes that, when corrected for the buoyancy of their submerged bases, the height of young oceanic volcanoes should vary as the square root of crustal age. A plot of the relevant observations confirms this relationship with a surprisingly low scatter of points about the straight line. Because the data obey the expected relationship, they implicitly support the assumptions upon which the relationship was derived. In other words, the results suggest that most volcano heights are indeed limited by isostasy rather than magma shortage. An increase in volcano height thus implies an increase in plate thickness, and a linear relationship between volcano height and the square root of age implies a similar relationship between plate thickness and square root of age. Hence there is support here for the Parker-Oldenburg model.

On the other hand, this does not mean that the constant thickness hypothesis may be dismissed without further consideration. For if plate thickness remains constant with age, the average plate density will increase with age, and this will also affect the height to which a volcano may grow according to isostasy. Vogt shows, however, that the height-age relationship expected in this case is quite unlike that actually observed.

Pulsing X-ray stars

from James Pringle

OF the sixty or so X-ray stars known in our Galaxy and in the Magellanic Clouds only a few have so far been found to pulse regularly. One, the Crab pulsar, has a pulse period of 33 milliseconds, as do its radio and optical counterparts, and is thought to be a rapidly spinning neutron star. The star's large energy output, several thousand times that of the Sun, derives from its rotational kinetic energy, the pulse period is observed to increase on a time scale of about a thousand years.

Another two, Centaurus X-3 and Hercules X-1, pulse with periods of 4.84 and 1.24 seconds respectively, but, although their X-ray luminosities are similar to that of the Crab pulsar, their pulse periods vary somewhat irregularly with no great evidence for overall speeding up or slowing down. Both these sources of X-rays are identified with optical stars. It is almost certain that the X-rays originate from a compact companion star, too faint to be visible optically, and that mass transfer from the 'normal' star on to its companion provides the source of energy. One strong possibility is that the compact star is a neutron star with a strong magnetic field (about 10^{12} Gauss). Material falling on to such an object glides along the field lines on to the magnetic poles where it yields about 10% of its rest mass energy on striking the stellar surface. The necessary quantity of energy released in such a small area must lead to high enough temperatures to provide X-ray emission. Rotation of the star, with its two X-ray hot spots, provides the pulse. Radiation produced in such a strong magnetic field should be highly polarised—an important observational test of this hypothesis.

An alternative possibility is that the compact object is a pulsating white dwarf star. For a neutron star, the gravitational energy released by the infall greatly exceeds the nuclear energy released by subsequent hydrogen burning. For a white dwarf of a solar mass, hydrogen burning releases 20 times as much energy as the infall, and even more for less massive stars. It has been suggested that this hydrogen burning could feed energy into pulsations of the star, these would then dissipate through shock waves in the stellar atmosphere. Preliminary work suggested that it was indeed possible to heat the white dwarf's atmosphere sufficiently to provide X-rays of sufficient energy and intensity. But in a recent, and more detailed, calculation, Katz and Salpeter (*Astrophysical Journal*, **193**, 429-436, 1974) conclude that these previous results were too optimistic and that any realistic calculation will give X-ray emission too low by many orders of magnitude.

The authors consider radial pulsations of the star which, at a specified layer below the photosphere, are assumed to be sinusoidal in velocity. They stress that the peak downward acceleration of such a motion cannot be taken to be greater than the effective surface gravity: this condition has been violated in previous work. Also, because in general the scale height in the atmosphere is much less than the radius of the star, there is an 'acoustic mismatch' (and thus a very weak coupling) between the low stellar eigen-

modes and atmospheric sound waves or shock waves. This mismatch can only be avoided if the luminosity of the star is close to the Eddington limit, in which case radiation pressure almost balances gravity and the atmospheric scale height is comparable with the stellar radius. In this event, however, the inverse Compton cooling (the sharing of energy between photons and electrons in the stellar atmosphere) produced by the stellar luminosity becomes most severe, and the shock heating of the atmosphere is suppressed. Compton cooling has been ignored in previous work and the present authors find it to be very important in damping atmospheric shock waves and removing energy from any shock heated material. These effects strongly inhibit the conversion of stellar oscillatory motion into multi-keV X-rays.

The relevance of the above constraints to the non-pulsing X-ray sources is as yet unclear, although the authors do indicate some misgivings about the white dwarf hypothesis as a whole. On the other hand, there has been a tendency among theorists to ignore the possibility of X-ray emission from white dwarfs and it is likely that further progress will be made in this field before very long.

Giant multipole resonances

from P. E. Hodgson

MANY years ago it was found that the cross sections for the radiative capture of protons by nuclei showed broad maxima at energies around 10 to 20 MeV. These persist throughout the periodic table, and accurate measurements showed that the energies of these states is given approximately by $63A^{-1/3}$ MeV.

Goldhaber and Teller (*Phys. Rev.*, **74**, 1046, 1948) explained these resonances as due to collective oscillations of the neutrons against the protons. This accounted both for their universal character and qualitatively for the variation of their energies from nucleus to nucleus. Subsequently a more detailed microscopic theory interpreted them as a quantum-mechanical superposition of particle-hole excitations giving a resonance of an E1 dipole character (Brown and Bolsterli, *Phys. Rev. Lett.*, **3**, 472, 1959; Elliott and Flowers, *Proc. R. Soc.*, **242**, 57, 1957).

Subsequently these dipole resonances were found to be excited in many other reactions, particularly by the inelastic scattering of electrons and protons (*Nature*, **246**, 250, 1973), and detailed analyses of these data allowed many of the properties of the resonances to be determined.

The identification of giant E1 dipole resonances naturally raises the question of the existence of other multipole resonances in nuclei, and over recent years the evidence for some of these has become more convincing. A giant quadrupole resonance several MeV below the giant dipole resonance was found by (e,e') experiments at Darmstadt (Pitthan and Walcher, *Phys Lett*, **36B**, 563, 1971) and confirmed by (p, p') analyses at Oak Ridge (Lewis, *Phys Rev Lett*, **29**, 1257, 1972). Further work at Sendai University (Fukada and Torizuka, *Phys Rev Lett*, **29**, 1109, 1972; Nagao and Torizuka, *Phys Rev Lett*, **30**, 1068, 1973) resolved the new resonance into three separate states, and also provided evidence for other resonances at 19 MeV (E3) and 22 MeV (E2) in ^{208}Pb .

The widths of all these resonances are subject to sum rules analogous to the Thomas-Reiche-Kuhn sum rule in atomic spectroscopy, and it was found that these resonances exhaust a large fraction of the appropriate sum rule, confirming their giant resonance character.

The analyses of proton inelastic scattering by von Geramb and colleagues (*Nucl Phys*, **A199**, 545, 1973) has provided particularly elegant and continuing evidence for some of the higher resonances. Fig 1 shows some of their results. This work enables the apparently irregular behaviour of the inelastic proton scattering at back angles from ^{12}C and ^{16}O to be accounted for, and also gives the parameters of several higher order resonances.

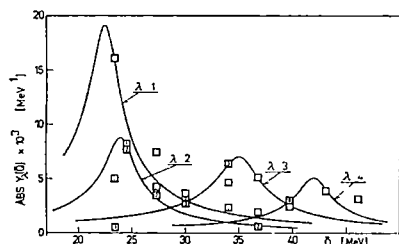


Fig 1 Multipole resonances in ^{16}O found from analysis of proton inelastic scattering. The numbers in squares and the values of λ are the multiplicities.

All this work has recently been strikingly confirmed by recent measurements of the inelastic scattering of 90 MeV electrons by lead and gold carried out by Pitthan and colleagues at Monterey (*Phys Rev Lett*, **33**, 849, 1974). Some of their results are shown in Fig 2, and it is clear that the electron energy spectrum has a definite structure that can be

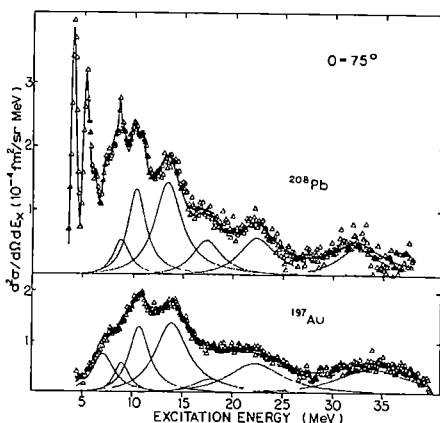


Fig 2 Spectrum of electrons after inelastic scattering at 75° from ^{208}Pb and ^{197}Au , after subtraction of background. The spectrum is analysed into the components due to the different contributing giant multipole resonances.

analysed into a number of separate components. The energies and widths of these resonances are given in Table 1, together with their multiplicities and whether they are isoscalar ($\Delta T = 0$) or isovector ($\Delta T = 1$), where T is the total isospin. It is notable that the widths of the resonances in ^{197}Au are significantly greater than the widths of the corresponding resonances in ^{208}Pb , this is to be expected since ^{197}Au becomes dynamically deformed when excited, whereas ^{208}Pb remains spherical.

Of the resonances in the table, the E2 resonance at 10.8 MeV is the familiar dipole resonance. The E3 and E2 resonances at 18 and 23 MeV respectively correspond to those found by the Sendai group.

The lowest E1, E2 and E3 resonances are probably the same as those found by von Geramb and colleagues, the widths are very similar although the energies are shifted somewhat, probably due to a different dependence on A through the periodic table. A distorted wave analysis confirms the E0 character of the resonance observed at 9.2 MeV. This is the first experimental indication of a giant monopole resonance and should make possible a determination of the nuclear compressibility. This identification is supported by evidence from (γ, n) spectra. The resonance at 33 MeV is new and the angular distribution for Au has an E0 (or possibly E2) character. This resonance might be the isovector monopole state proposed by Bohr and Mottelson (*Nuclear Structure*, vol 2, 1972), but an accurate assignment is difficult.

Table 1 Characteristics of the giant multipole resonances found from analyses of inelastic scattering of 90 MeV electrons by ^{197}Au and ^{208}Pb .

$E_x A^{1/3}$	EL	ΔT	^{197}Au		^{208}Pb	
			E_x	Natural width (MeV)	E_x	Natural width (MeV)
53	E0	0	9.2	2.2 ± 0.5	8.9	1.8 ± 0.5
63	E2	0	10.8	2.9 ± 0.2	10.5	2.8 ± 0.3
81	E1	1	14.0	4.5 ± 0.2	13.6	3.9 ± 0.1
105	E3	0	18.0	5.1 ± 0.7	17.5	4.2 ± 0.7
133	E2	1	23.0	7 ± 1	22.5	5 ± 1
195	E0	1	33.5	10.5 ± 2	33.0	6 ± 1

Persistent superfluid flow

from P V E McClintock

In a recent communication to *Physical Review Letters* (**33**, 1073, 1974), Gal-kiewicz and Hallock report the first really satisfying demonstration of a persistent superfluid current in a helium film. In their experiment, which was carried out at the Amherst campus of the University of Massachusetts, they started the ^4He film moving at a velocity of about 5 cm s^{-1} around a closed path some 40 cm in length, and were then able to show that the energy of flow did not measurably diminish over a period of several hours.

Like other liquids, helium tends to form a thin film up the walls of the vessel which contains it, held in place by the Van der Waals attractive force between the helium atoms and the atoms of the wall. In contrast to other liquids, however, whose films usually evaporate a short distance above the free surface and which are constrained by viscous forces to move at very small velocities, the helium film can indulge in superflow, that is, it can flow at several cm s^{-1} without any dissipation of its energy of flow. Thus, any evaporation of the film is quickly replaced by superflow from the bulk liquid. All surfaces in contact with superfluid helium will therefore be completely covered with a relatively thick (typically 200 Å) film of helium. It is superflow in the film which is responsible for the well known and dramatic phenomenon of the beaker of helium which empties itself while remaining upright; the liquid simply syphons itself out through the channel provided by the film.

The non-dissipative nature of film flow has been fairly convincingly established by experiments where a flow from one vessel to another was maintained without any measurable driving force. Yet, apart from some interesting work in compressed powders and other porous media, no previous attempt to set up a persistent flow of the film around a closed path has been successful.

The most impressive of these earlier investigations was probably that of Wang and Rudnick of the University of California at Los Angeles, described in the recently published *Proceedings of the 13th International Conference on Low Temperature Physics* (edit by K. D. Timmerhaus *et al.*, vol 1, 239, Plenum Press, 1974). Their substrate consisted of a glass cylinder which could be rotated, and they arranged to be able to measure the times of flight around the cylinder of surface waves on the adsorbed helium films (known as third sound) either with or against

the expected persistent current. The velocities of third sound relative to the substrate in the two directions would have been different in the presence of a persistent flow, but no such phenomenon was observed during a series of very careful experiments. In fact, the authors concluded that any such flow, if it existed, was occurring at velocity of less than 1 cm s^{-1} .

In the Amherst experiment both the geometry and the method of initiating the film superflow were entirely different. Rather than on the outside of a cylinder, the film was made to flow along the inside of a 37 cm length of 1.37 mm diameter stainless steel tube, closed on itself so as to form a continuous loop. Also connected to the loop, by separate short copper tubes, were the annular spaces in two pairs of coaxial cylinder capacitors, c_1 and c_2 . On the 12 cm length of the loop between these two connections was wound a heater which, when energised, evaporated the film and prevented it flowing past that point, thus acting as a sort of switch for the superflow. At the beginning of an experiment c_1 and c_2 were partly filled with superfluid helium so that the tubes above them and the stainless steel loop were covered with the film. In coming to equilibrium the liquid levels were, of course, equalised by film superflow. By measuring the capacities of c_1 and c_2 it was possible to determine to a very high precision the amount of dielectric, and thus the level of the liquid helium inside each of them.

To initiate film flow, the heater was energised and a d.c. voltage was applied for a short while across c_2 . The immediate result was a flow of the film out of c_1 , around almost all of the stainless steel loop (except, of course, the short length between c_1 and c_2 which incorporated the heater), and into c_2 . A well known property of film flow between reservoirs is the tendency to overshoot when the levels equalise, resulting in slowly damped oscillations with a period of several seconds. Thus, the disturbance of the levels in c_1 and c_2 was followed by an oscillatory flow between them through the connecting helium film. By de-energising the heater at an appropriate moment the loop circuit was completed for superflow and, though the level oscillations gradually decayed away, it was hoped that a persistent current might be left in the loop.

To test for the presence of a persistent current after a lapse of time, the heater was again energised, thus opening the loop circuit, so that any kinetic energy flow in the film would have to go into depressing the level in the capacitor on the upstream side of the heater and therefore exciting oscillations once more. In practice level

oscillations did indeed occur, even after a lapse of as long as seven hours, and the amplitude of the oscillations did not vary to a measurable extent with the waiting time. The implication must be that Galkiewicz and Hallock had succeeded for the first time in setting up a persistent state of flow around the loop, quite analogous to the persistent electrical current which can be set up in a closed circuit of superconductor in each case there is a macroscopic flow of particles which apparently persists indefinitely without any dissipation of energy.

Why, on the other hand, was Wang and Rudnick's carefully designed experiment a failure? The reason is not yet known but, on the basis of the present experiment, it is unlikely to be connected with momentum exchange between the flowing film and the helium vapour with which it is in dynamic equilibrium, as had been suggested earlier. This same situation also pertained in the successful Amherst experiment. It is to be hoped that further developments of Galkiewicz and Hallock's new technique will yield the answer to this and other problems connected with film superfluidity.

Changes in enzyme activity during plant photomorphogenesis

from our Plant Cell Physiology Correspondent

PROFOUND changes in the activity of many plant enzymes occur during photomorphogenesis, the response of dark-grown plants to light. Most changes are brought about by red or far-red light acting through the phytochrome system, in some instances a photoreceptor absorbing blue light is involved. Typically the activity of enzymes already present in dark-grown plant tissue is stimulated above the basal level within hours. Although other phytochrome-controlled responses occur more rapidly, this system is very convenient for studying the regulation of enzyme activity during plant development. Using the density-labelling technique several groups have obtained evidence suggesting that at least two mechanisms are involved—*de novo* enzyme synthesis and the activation of pre-existing inactive enzymes.

The essence of the density-labelling technique is simple. In order to test whether an increase in enzyme activity in response to a given stimulus is due to synthesis, a heavy isotope such as ^{18}O , ^3H or ^{15}N is briefly supplied to the

tissue at the same time as the stimulus. A protein extract is later prepared from the tissue and fractionated by buoyant density in CsCl using an ultracentrifuge. If the degree of labelling of the enzyme being studied is significantly greater in the stimulated tissue than in control tissue this indicates an enhanced rate of *de novo* synthesis.

Attridge, Johnson and Smith (*Biochim Biophys Acta*, **343**, 440, 1974) have studied the phytochrome-controlled increase in activity of phenylalanine ammonia-lyase (PAL) in mustard cotyledons. They have shown, using $^3\text{H}_2\text{O}$, that PAL and acid phosphatase become density labelled in cotyledons of both irradiated and dark-grown plants, indicating that enzyme synthesis is taking place in all cases. The degree of density labelling of acid phosphatase is unaffected by irradiation, whereas if PAL labelling is measured during the time that the enzyme activity increases in response to light the density labelling of the enzyme is actually lower than in dark grown plants. The authors suggest that the increase in enzyme activity detected in the light is due to the activation of previously synthesised enzyme, although a decrease in the rate of degradation of the enzyme could also be involved. Similar results were obtained for the blue-light controlled increase in PAL activity in gherkin hypocotyls (Attridge and Smith, *Biochim Biophys Acta*, **343**, 452, 1974) and the authors cite evidence for the presence of a substance in this tissue which reversibly inactivates PAL.

Another enzyme that increases in activity in irradiated mustard cotyledons is ascorbic acid oxidase. Attridge (*Biochim Biophys Acta*, **362**, 258, 1974) has shown that when dark-grown plants are transferred to $^3\text{H}_2\text{O}$ and irradiated with far-red light, the density of the enzyme increases to the maximum observed within six hours (much more rapidly than the dark control). This evidence strongly suggests that ascorbic acid oxidase, unlike PAL, is synthesised more rapidly in response to light. Increased ribonuclease activity in lupin hypocotyls also seems to be due to phytochrome-controlled stimulation of enzyme synthesis according to Acton and Schopfer (*Biochem J*, **142**, 449, 1974). These workers were able to demonstrate a greater increase in density of the enzyme in illuminated plants compared to the dark-grown controls. Band-broadening of the enzyme peak in CsCl also provided evidence for the presence of a population of differently deuterated enzyme molecules—to be expected if the density marker takes a significant time to equilibrate with the amino acid pool. The regulatory mechanisms governing the synthesis of these enzymes remain to be determined.

articles

Earliest record of man's presence in Britain

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Middle Pleistocene deposits near Westbury-sub-Mendip have yielded flints which pose problems in the local geological context. Some of the flints show signs of having been worked by man and as such they may be artefacts. The flint-bearing layers contain a rich mammalian fauna of Cromerian age, which suggests that the artefacts may mark the earliest record to date (~400,000–500,000 yr BP) of man's presence in Britain.

WORKED flints have been recovered from cave sediments of late Cromerian age near Westbury-sub-Mendip, Somerset. The deposits, which may provide the earliest record yet of man's presence in the British Isles were first noticed in 1969 (see refs 1 and 2). They are exposed in a working Carboniferous Limestone quarry (ref IT 506504) between 213 m (700 feet) and 244 m (800 feet) or on the southern plateau edge of the Mendip Hills. They infill, to ground level, what is believed to be part of a former cairn system. The present exposure, in the unworked northern face of the quarry, is 90 m wide, and nearly 30 m high.

Stratigraphy

The main stratified sequence¹ (Fig. 1) is no longer visible, and much of it has been blated out in the interests of safety and has been lost forever. The beds were well stratified and about 10 main layers, grouped into two main divisions, could be distinguished, in which three separate mammal faunas have been recognised (Table 1).

The sands and gravels of the Siliceous Group (Table 1) are waterlaid deposits, comprising material that is largely foreign to the area. Rare, angular fragments of Carboniferous chert occur, but Carboniferous Limestone, if it was ever present, has been removed by solution. The foreign constituents are thought to be derived from former cover sediments, but no flint has been found in these, which emphasises that the latter is foreign to the overlying Calcareous Group. A sparse mammal fauna has been collected from the gravels and although it is apparently derived it is no earlier than late Lower Pleistocene.

The Calcareous Group comprises conglomerates and breccias made up of rounded to angular fragments of Carboniferous Limestone, with variously coloured matrices of silt and clay. Well stratified horizons occur only in the central area of the infill, and these yield both the flint and the richest remains of large fossil mammals. The bulk of the mammal finds have so far come from beds 3, 4 and 5 (Fig. 1), which are regarded as downwash accumulations from a former, upstream 'den'. Unfortunately, it is this valuable stratigraphical sequence which has been destroyed. Laterally to the west of this sequence is the Roent Earth (Table 1) and on the east side is an extensive sequence of breccia deposits which are

rich in fossil mammal material, but which have not yielded flints so far.

Most of the rock constituents of the Calcareous Group are Carboniferous Limestone, though there are indications that some of the limestone is not local, but has been transported into the infill from the surrounding district. The only other rock types in this group are the flints, and three rounded pebbles of ironstone, all found in the central stratified sequence of the Calcareous Group.

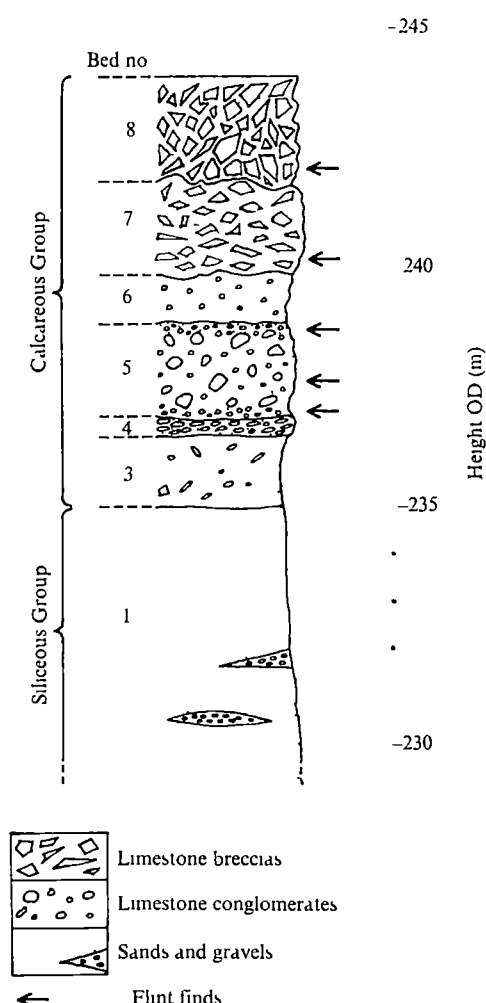


Fig. 1 Stratigraphical section of the upper part of the main layered sequence, visible up to July 1974

Fauna and age

The mammal faunas have been used to date the deposits and experimental radiometric work on stalagmite samples from Westbury is under way (D Ford, personal communication).

The fauna of the Calcareous Group comprises predominantly bear remains, probably all *Ursus deningeri* Reichenau, the bones and teeth of which represent individuals of all ages, indicating that the species was a resident of the area. Shelter was probably available in a former upstream, cavern area, which provided a den for bears and occasional living quarters for other hunting animals. A large proportion of other carnivores which are of Cromerian aspect are represented in this fauna, of which the small wolf *Canis lupus mosbachensis* Soergel is very common. Remains of the sabre-toothed cat *Homotherium latidens* Owen, have been found in a number of horizons, and the first British occurrence of the extinct dhole *Xenocyon lycaonoides* Kretzoi, and the 'European jaguar' *Felis gombaszoegensis* Kretzoi, are recorded here. Herbivores form a proportionately small part of this fauna, but they include the rhinoceros *Dicerorhinus etruscus* Falconer which is an important faunal element in indicating a Cromerian age.

Few mammal remains have been collected from bed 8 mainly because it is not easily accessible. The contemporary Rodent Earth is physically separate from the main outcrop of the Calcareous Group, but apparently belongs to it on the basis of its fauna. The fauna of the Rodent Earth comprises predominantly small mammals, most of which are voles, *Pitymys gregaloides* Hinton being the most common. The first British occurrence of the vole *Phomys episcopalus* Méhely is recorded here. Both of these species indicate that this fauna is pre-Holsteinian (pre-Hoxnian). The abundance of *Arvicola cantiana* Hinton and absence of *Mimomys*, suggests strongly, however, that this fauna is later than the Upper Freshwater Bed of West Runton³ (Cromerian Zone II⁴), as *Mimomys* is believed to be the direct ancestor of *Arvicola*^{5,6}.

Table 1 Main divisions of the early Middle Pleistocene sequence

Calcareous Group (cave conglomerates and breccias)	Rodent Earth Fauna
Siliceous Group (waterlaid sands, silts and gravels)	Calcareous Group Fauna
	Siliceous Group Fauna

The faunas of the Calcareous Group and the Rodent Earth correlate well with the European sites at Mosbach, Mauer, Hundsheim and Tarko, which are usually considered to be between Cromerian (*sensu stricto*) and Elsterian in age. Correlation with the type Cromerian of the Cromer Forest Bed Series³ is difficult because most of the mammalian material of the type area was collected in the nineteenth century, and records of localities and stratigraphical position are often lacking. There are, however, good records from the West Runton Upper Freshwater Bed⁴. By comparison, the Westbury faunas seem to be younger, and for this reason are here termed 'late Cromerian'. It is possible that they are of about the same age as the younger fauna from Ostend, near Bacton⁴, as *Arvicola cantiana* is common to both sites. Koenigswald⁶ regards the faunas from Mosbach, Mauer, Hundsheim and Tarko as later than the Upper Freshwater Bed but earlier than Holsteinian (Hoxnian). It would seem that these sites, and the Westbury site as well, show clear faunal distinctions from the Holsteinian, and to some extent from the Cromerian (*sensu stricto*), yet these faunas are themselves temperate. It may be that they are separated from the Cromerian (*sensu stricto*) by a climatic barrier such as a cold phase, or a fully glacial phase (see ref 7). Preliminary palynological data (R N L B Hubbard, personal communication) on a Westbury sample from the level of bed 4, indicates that this is zone II of an interglacial. It is hoped that future work on the palynology, fauna

and sedimentology will elucidate the problem of whether a previously unrecognised temperate age is represented in Britain between the Cromerian (*sensu stricto*) and the Hoxnian.

Flints

Flints occur at five horizons within the Calcareous Group (Fig 1). The flint is white and usually rotted, often to the extent that it breaks down easily to fine powder. It is non-calcareous, shows conchoidally fractured faces, and contains

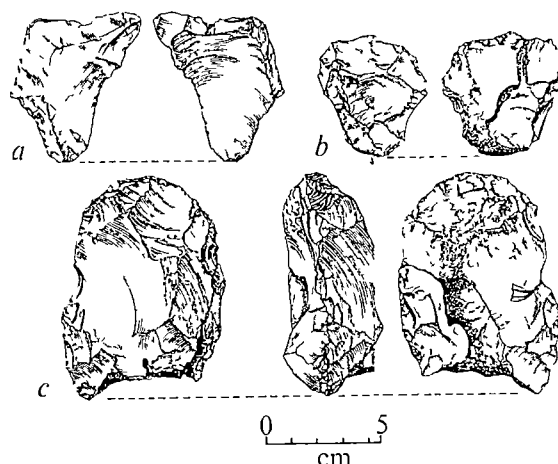


Fig 2 a, struck flake, from bed 5, b, rude bifacial flint from bed 5, worked from a flattened pebble, c, Ovate, bifacial worked flint from bed 8

Upper Cretaceous foraminifera. It occurs most commonly as angular or subangular pieces up to cm in size, in bed 5 they occurred as frequently as one in every 8,000 cm³. Sizes between 3 cm and 9 cm are rare (Fig 1). Despite the chemical breakdown in most of the flints, the larger specimens are well preserved and show few signs of rounding. Even under microscopic examination the ridges between the flake scars are sharp and fresh, indicating that the flints have not travelled far. Only around the leading edges of the large flints do signs of wear and abraded occur.

Five of the flints show apparent signs of human workmanship. The oldest two are from bed 5, one is a flake (Fig 2a) and the other is a small bifacial implement (Fig 2b) which retains part of the original surface of the flattish pebble from which it was fashioned. The largest and most diagnostic implement (Fig 2c) is from bed 8, it is thick bifacially worked ovate. The two bifacially worked pieces of flint (Figs 2b and c) are probably Acheulian (K P Oakley, personal communication). As the associated fauna has been identified as Cromerian, these implements must rank among the earliest Acheulian artefacts known in Britain, presumably comparable with the so-called Abbevillian in the 45 m terraces of the Somme (K P Oakley, personal communication). The preservation of the flints, and the fact that sediment samples (from the level of bed 4) broken down for pollen, contained abundant microscopic charcoal fragments (Hubbard, personal communication), indicate that an occupation site may have existed in the former cavern area.

The new finds at Westbury support suggestions⁸ of an early date for the worked flints from the basal breccia at Kent's Cavern, Torquay⁸, because it is now clear that man was present in southern Britain much earlier than had been supposed. Unfortunately, the mammal fauna from these basal breccias is too poorly known at present to allow reliable correlation with the Westbury deposits, though the presence of *Arvicola*

and *Pitymys gregaloides* at both sites is highly suggestive of such a correlation

The earliest occupation sites in Europe have, from a consideration of their mammal faunas, been described broadly as Cromerian⁹. Those with good artefactual evidence, within a well defined stratigraphy, and with good faunal associations are extremely rare, they include, however, Vértesszölös in Hungary (considered to be Elsterian in age), and Vallonnet in France⁹. Although Westbury does not seem to represent the immediate area of an occupation, there is evidence that an occupation site may have existed very close to the present location of the artefacts. This, and the fact that Westbury displayed a very well defined stratigraphy with very rich and abundant faunal associations, ranks Westbury among the most important Pleistocene sites in Europe with evidence of man.

Although the valuable flint bearing strata have been blasted

away, it is hoped that more finds will be made by examining all of the fallen material, new flint bearing layers may become exposed as a result of future excavations.

I thank Westbury Quarry (Mixconcrete Aggregates Ltd) for permission to study deposits in the quarry, and Professor D T Donovan, Dr J E Robinson and Dr A J Sutcliffe for criticism. I thank Miss M O Miller (British Museum) for drawing the flints.

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Birefringence experiments on isolated skeletal muscle fibres suggest a possible signal from the sarcoplasmic reticulum

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When stimulated by external electrodes, a frog muscle fibre bathed in hypertonic Ringer gives a birefringence signal consisting of three components. The small first component is due to the action potential of the surface membrane, and the much larger second component may be related to voltage change across the sarcoplasmic reticulum associated with the release of Ca²⁺. The third component is of uncertain origin.

SEVERAL investigators^{1–8} have described light signals which can be detected during the twitch of vertebrate skeletal muscle. These signals are usually measured as changes in light intensity, but depend on various optical mechanisms, including absorption and/or scattering^{1–5}, fluorescence^{4,6} and birefringence^{4,7,8}. In the hope of learning more about the very early events underlying the activation of contraction, we have carried out birefringence experiments on single muscle cells. The major problem in optical experiments on excitable tissue is often not so much to find light signals but to identify as precisely as possible the subcellular events which cause them. So far this effort has been less successful in muscle than in nerve.

Cohen *et al.*⁹, in birefringence experiments on the squid giant axon, showed that signal averaging techniques could detect a small decrease in light intensity coincident with the action potential. This signal had a peak size of about 1×10^{-5} (maximum change in light intensity during the spike divided by resting light intensity). Voltage clamp experiments¹⁰ provided good evidence that the birefringence signal was principally related to potential change across the surface membrane, and showed that the light signal followed the voltage change with a time constant of tens of microseconds.

Unlike nerve, a skeletal muscle fibre has in addition to the surface membrane two well developed membrane systems

thought to be important in the activation of contraction. These are (1) the transverse tubular membranes (T-system), which has in frog sartorius muscle 5–10 times as much membrane area as the surface^{11,12}, and (2) the sarcoplasmic reticulum (SR), which has in frog 75–150 times as much membrane area as the surface¹¹. The physiological roles of these membrane systems are, in outline, fairly well understood. The action potential on the surface membrane depolarises the T-system within the fibre^{13,14}, which in turn triggers the release of Ca²⁺ into the myoplasm from its internal storage site, the sarcoplasmic reticulum¹⁵. The increased myoplasmic Ca²⁺ concentration then activates the contractile proteins¹⁶.

If the membrane systems of muscle, like the squid giant axon, give birefringence signals dependent on membrane potential, these signals might be considerably larger in muscle, because of the greater quantity of membrane involved. Carnay and Barry⁴, in birefringence experiments on muscle bundles of about 50 fibres, found a relatively large decrease in light intensity (peak size about 2×10^{-4}) beginning coincident with the extracellularly recorded action potential. As this signal was about 20 times larger than that for the action potential in squid, it seems unlikely that all of it can be attributed directly to depolarisation of the surface membrane. Because all fibres in a bundle or whole muscle will not give the same signal at the same time, we decided to study muscle birefringence signals using the isolated single fibre preparation.

Single fibres from the semi-tendinosus or ilio-fibularis muscles of frogs (*Rana temporaria*) were isolated and stretched around a small vertical glass post, to minimise vibrational motion and hence optical noise, in a glass-bottomed Perspex chamber. One tendon end was attached to the arm of an RCA 5734 mechano-electric transducer for monitoring isometric tension. The chamber was then mounted on the stage of a Reichert Zetopan polarising microscope with a $\times 40$ water-immersion lens (Zeiss, N.A. 0.75) for optically recording from a 500 μ m length of the fibre. Two platinum wire electrodes brought close to the fibre delivered the stimulus pulse (duration 0.2–0.5 ms).

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Only those fibres giving an all-or-nothing response to external stimulation were used for experimentation. The illuminating light, from a 100 W FCR tungsten-halogen bulb, traversed a heat filter and two plane polarisers, one preceding and one following the fibre in the light path. To maximise the size of birefringence signals from subcellular structures with optical axes either parallel or perpendicular to the fibre axis, one polariser was oriented at -45° and the other at $+45^\circ$ with respect to the fibre axis. The light detector, a PV-100 photodiode (EG & G Inc.), produced an output current that was a linear function of light intensity transmitted by the second polariser. This output was converted to a voltage signal by means of a conventional operational amplifier circuit, amplified by a 3A9 amplifier in a Tektronix 565 oscilloscope and fed to one of two signal averaging computers, CAT model 1000 (Technical Measurement Corp.) or Nicolet 1072 (Nicolet Instrument Corp.). The response of the light-recording system to a step of light was 70% complete within 80 μ s. A standard Ringer solution was used: 120 mM NaCl, 2.5 mM KCl, 1.8 mM CaCl_2 , buffered with 2.15 mM Na_2HPO_4 and 0.85 mM NaH_2PO_4 to a pH of 7.1 (ref. 17). For many experiments the Ringer was made hypertonic (see below) by increasing the concentration of NaCl. The temperature for most experiments was between 19° and 24° C. In voltage-recording and current-passing experiments, glass microelectrodes with a right-angle bend to fit under the microscope objective were used, in order that the optical signal could be recorded simultaneously with intracellular electrical measurements.

Birefringence signal without fibre movement

During a twitch in normal Ringer, gross movement of the fibre gives rise to very large optical signals, of the order of 10^{-2} even in highly stretched fibres. Bathing a fibre in 2.2T hypertonic Ringer (2.2 times the standard tonicity), however, greatly reduces or eliminates fibre movement while the surface membrane still gives normal or nearly normal action potentials¹⁸. In fibres in which 2.2T hypertonic Ringer reduced the twitch tension response essentially to zero, three components of the optical signal were usually seen, as Figs 1 and

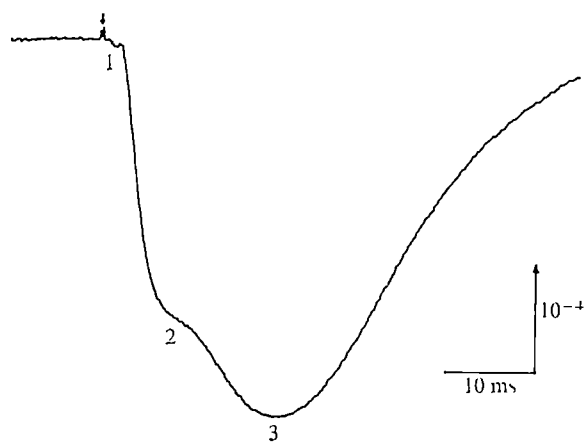


Fig. 1 Three-component birefringence signal in response to external stimulation from a muscle fibre in 2.2T hypertonic Ringer (270 mM NaCl). The trace is an average of 100 all-or-nothing responses. The peaks of the three components are labelled, the first component begins after a small conduction delay following the stimulus artifact (arrow). The vertical calibration gives the ratio of the change in light intensity to the resting light intensity, normalised with respect to a single sweep. A deflection in the downward direction represents a decrease in light intensity. Optical signal d c coupled, optical recording 2 mm from the stimulus cathode, fibre diameter 85 μ m.

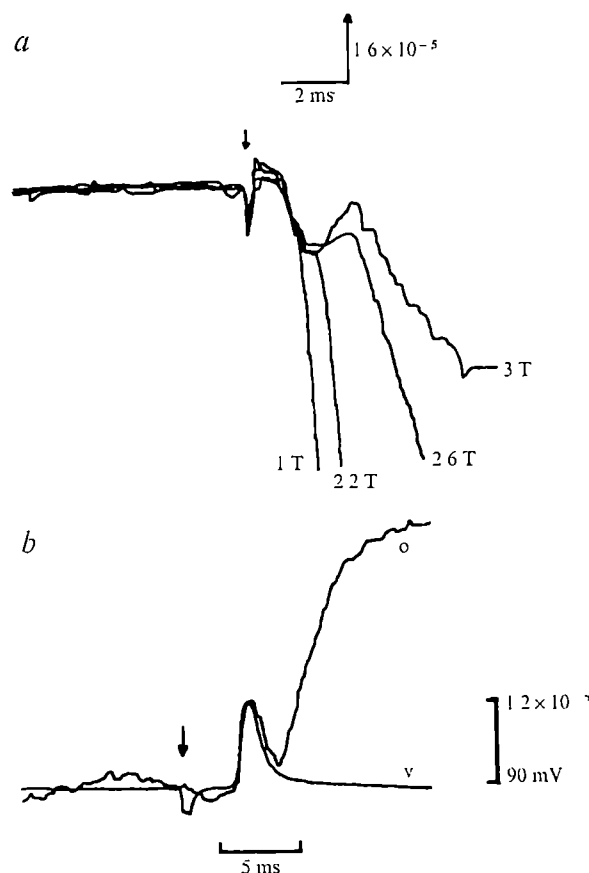


Fig. 2 *a*, Optical recordings from the same fibre in Ringer solutions of various tonicities superimposed by lining up the stimulus artifact (arrow). 1T, 120 mM NaCl, 2.2T, 270 mM NaCl, 2.6T, 320 mM NaCl, 3T, 370 mM NaCl Ringer. Each tracing is the average of 64 sweeps, which were taken in the order of increasing tonicities. Following the record in 3T Ringer, the fibre still gave an all-or-nothing response when returned to normal Ringer, with its average for 64 sweeps falling between the 1T and 2.2T tracings shown. Optical recording 1.5 mm from stimulus cathode, optical signal a c coupled, fibre diameter 85 μ m. *b*, Optical (o) and intracellular voltage (v) recordings taken simultaneously in 3T Ringer (370 mM NaCl). The optical tracing has been inverted and scaled to facilitate comparison with the action potential. The upper vertical calibration refers to the optical trace and the lower to the potential trace, each of which is an average of 300 sweeps. Resting potential of the fibre varied between -70 at the beginning of the average to -65 at the end. In agreement with results reported by others, we found that strongly hypertonic solutions tended to depolarise the fibres. Fibre diameter 65 μ m, 7 mm from the stimulus cathode, optical a c coupled, voltage d c coupled.

3b show (1) a small, early shoulder which occurred just as the optical trace departed from the baseline and which reached a distinct peak (Fig 1) or a plateau (Fig 3*b*) in 1–2 ms (this component is too small to be distinguished in a single sweep, and in a bundle or whole muscle would probably be difficult to resolve, even with signal averaging, because of differences in conduction velocity among fibres), (2) a relatively large signal, which reached a plateau (Fig 1) or a distinct peak (Fig 3*b*) 5–7 ms after the beginning of the optical signal (large enough to be seen easily in a single sweep), and (3) a more slowly developing signal which reached a peak 15–50 ms after the stimulus. As determined in other experiments in hypertonic Ringer, the first and second components propagate with a velocity appropriate to a muscle action potential at room temperature (about 1.5–2 mm ms⁻¹) and were seen in fibres with a twitch tension response less than 0.3 g cm⁻². These signals were primarily due to a birefringence mechanism, since they could be converted from a decrease in light intensity to an

increase in light intensity by adding an appropriate amount of optical retardation in series with the light path using a compensator⁹

First component

An early shoulder (first component) similar to that of Figs 1 and 3b was usually seen with signal averaging in hypertonic Ringer but only occasionally seen in normal Ringer. An explanation for this is that hypertonicity slows the development of the second component while leaving the first relatively unchanged. Thus the two components become separated in time. This is shown in Fig 2a, in which optical recordings from the same fibre in Ringer solutions of increasing tonicities have been superimposed by lining up the stimulus artefact. In normal Ringer there is an extremely fast departure of the optical trace from the baseline with no evidence of an early shoulder, whereas in 3T Ringer an early signal about 1.6×10^{-5} (peak size) with a time to peak of 1 ms is revealed. In Ringer solutions of intermediate tonicities, the first component is seen at intermediate states of development, before being obscured by the rising phase of the second component.

It is interesting to know the timing of the first component in relation to the surface action potential. This was determined for the fibre of Fig 2b in 3T Ringer by simultaneously recording the optical and intracellular voltage signals for an average of 300 sweeps. The vertical gains have been adjusted to match peak size and the optical trace inverted to facilitate comparison. The two traces superimpose very closely until late in the falling phase of the action potential. It seems reasonable therefore to attribute most of this early distinct signal and the shoulder seen in less hypertonic Ringer to the voltage change across the surface membrane.

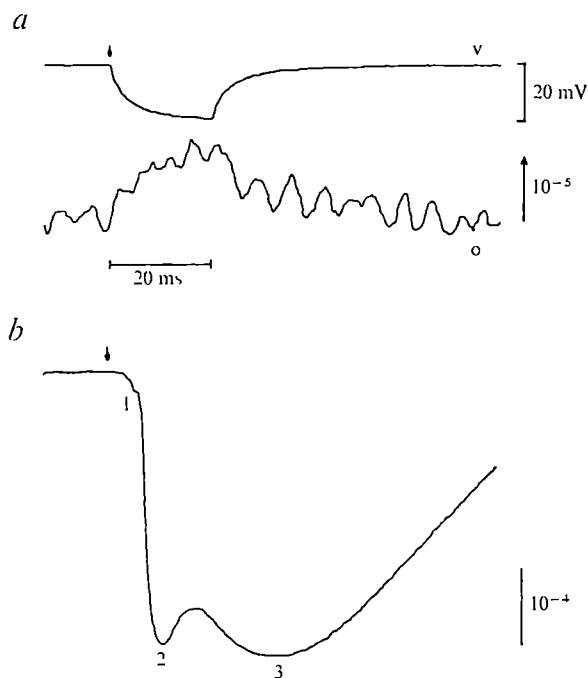


Fig 3 a, Current-passing experiment showing the simultaneously recorded optical (o) and intracellular voltage (v) responses in 2.2T Ringer (270 mM NaCl) for an average of 256 sweeps per trace. At the time indicated by the arrow a 20-ms step of inward current was passed across the cell membrane through a second intracellular electrode (current trace not shown). Resting potential of the cell was -80 mV. Fibre diameter $90 \mu\text{m}$, optical a c coupled, voltage d c coupled. b, Three-component signal in response to external stimulation, taken just before the fibre was impaled for the current-passing experiment shown in (a). Optical a c coupled, 8.5 mm from cathode, 64 sweeps. The same time calibration applies to both parts of the Figure.

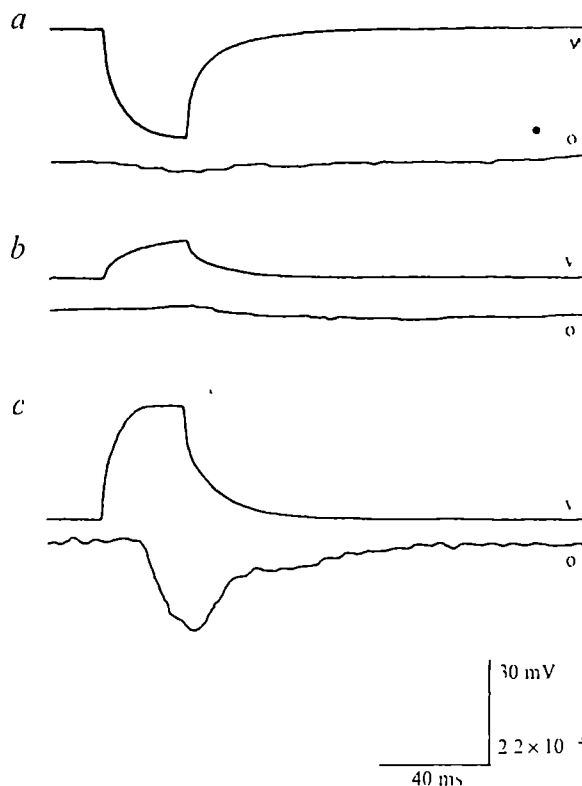


Fig. 4 Current-passing experiment in 2.2T hypertonic Ringer with TTX added (10^{-8} g ml^{-1}). Voltage (v) and optical (o) responses were simultaneously recorded during (a) a 30 mV hyperpolarising step, (b) a 10 mV depolarising step, and (c) a 30 mV depolarising step. Resting potential was -68 to -66 mV. Fibre diameter $90 \mu\text{m}$, optical a c coupled, voltage d c coupled, 32 sweeps per trace.

Second component

In contrast to the first component, the characteristics of which appear to be relatively independent of tonicity, the appearance of the second component is strongly dependent on tonicity. As the tonicity is raised, time to peak is increased and rate of rise (Fig 2a) and peak size are reduced. For example, the time to peak was often extended to 8 or 10 ms and peak size typically decreased by a factor of 5–10 in going from 2.2T to 3T Ringer. On the other hand, in measurements in normal Ringer directly over the site of action potential initiation, a much larger decrease in light intensity began immediately after the stimulus, occasionally starting with a distinct first component, and reached a plateau or peak 2–5 ms later. In normal Ringer the second component had a peak size of 1.3×10^{-3} , about 50 times larger than the second component seen in 3T Ringer.

Since fibre movement itself is a strongly graded function of tonicity, the question of whether the second component is related to fibre movement should be considered carefully. Clearly the second component is not due to simultaneous movement of the whole fibre, because it propagates with the speed of the action potential. When the Ringer was made sufficiently hypertonic so that twitch movement was essentially eliminated, the second component propagated throughout the fibre. In normal Ringer, however, gross fibre movement begins well before the action potential can propagate over all of the fibre. In this situation the propagating second component became obscured by the movement artefact after travelling some distance from the stimulus cathode. With signal averaging in normal Ringer, latency relaxation (the earliest fibre movement detectable by the 5734 transducer) was seen to begin about 2 ms after the stimulus. By this time the second component recorded

adjacent to the stimulus cathode was near or at its peak in many fibres, which also confirms that the second component is not due to gross fibre movement. The second component therefore reflects a propagating change beginning just after the surface action potential, possibly a step linking excitation to contraction.

To determine what fraction of the second component might be attributable to depolarisation of T-system membranes alone, current-passing experiments of the type illustrated in Fig. 3 were done. Figure 3*a* shows the optical signal resulting from a current-passing experiment on a fibre in 2.2T Ringer. The fibre was impaled with two separate electrodes, one for passing current and one for recording potential. At the time indicated, a step of hyperpolarising current was passed across the cell membrane and the voltage and optical responses were recorded and averaged. Although the signal-to-noise ratio was rather poor because of the small signal and mechanical vibrations arising from the two intracellular electrodes, the resulting optical signal for an 18 mV hyperpolarisation had a peak size of about 1×10^{-5} . Although not convincingly resolved in Fig. 3*a*, the time-course of the optical signal recorded in other experiments giving larger voltage and optical responses followed voltage and not current. Since the current step was sufficiently long to ensure that the potential across the T-tubular membranes was within a few per cent of the surface potential, the optical signal in Fig. 3*a* probably reflects voltage changes across both surface and tubular membranes. On this basis, one would not expect an action potential in the T system, causing a tubular membrane potential change of perhaps 100 mV¹⁹, to produce a decrease in light intensity for this cell larger than about 5 to 6×10^{-5} . Therefore, T-tubular depolarisation alone would explain only a small fraction of this fibre's second component, which had an amplitude of 3.5×10^{-4} in 2.2T Ringer (Fig. 3*b*).

Not all current-passing experiments produced a birefringence signal with the polarity indicated in Fig. 3*a*, the polarity to be expected if T-tubular membrane always gives an optical signal with the same sign as that from the surface membrane. Fibres of larger diameter occasionally gave signals of opposite polarity (a decrease in light intensity in response to hyperpolarising steps and an increase in light intensity in response to small depolarising steps), while the first and second components in response to external stimulation showed the usual decrease in light intensity. Although this phenomenon is not completely understood, the results from the current-passing experiments in both kinds of fibres in both 2.2T and normal Ringer support the conclusion that the mechanism underlying the second component is not primarily potential change across the membranes of the T-system.

To investigate further the optical signal resulting from a controlled change of surface and tubular membrane potential, current-passing experiments were carried out using tetrodotoxin⁶ (TTX) (10^{-6} g ml⁻¹), which eliminates regenerative depolarisation of surface and tubular membranes. Figure 4 shows such an experiment for a fibre in 2.2T hypertonic Ringer. At the lower optical gain used, little or no light signal was seen during a current step in the hyperpolarising direction (Fig. 4*a*) or a small current step in the depolarising direction (Fig. 4*b*). A large birefringence signal developed, however, with a moderate-sized depolarisation into the voltage range where contraction is normally activated (Fig. 4*c*). The highly nonlinear dependence of this signal on membrane potential makes it improbable that the signal can be explained simply in terms of voltage change across surface and T-tubular membranes. In other TTX experiments, using larger depolarising steps of short duration, the optical signal was larger and reached an earlier peak, resembling in size and time course the second component seen in 2.2T Ringer following the action potential. This similarity suggests that the second component is caused by the mechanism that gives rise to the optical signal illustrated in Fig. 4*c*, and therefore that the second component is caused by a mechanism which is strongly regulated by surface or T-tubular membrane potentials in the contractile range.

Second component and sarcoplasmic reticulum

The second component is $3\text{--}5 \times 10^{-4}$ in 2.2T Ringer (about 30 times the size of the optical signal attributed to the surface action potential) and in normal Ringer is 1 to 3×10^{-3} (about 150 times that of the surface action potential). If it originates directly from a membrane potential change, only the sarcoplasmic reticulum would seem to have sufficient membrane area to account for its size. Furthermore, it is reasonable to speculate that there is an SR potential change occurring at the time Ca²⁺ is released to trigger the activation of contraction. For example, if Ca²⁺ release were the result of a Ca²⁺-specific permeability increase in the sarcoplasmic reticulum membrane, the SR might undergo a sizeable potential change within a few ms of the action potential on the surface membrane. During a twitch at room temperature, a muscle fibre typically begins the rapid production of positive tension 3–6 ms after initiation of the action potential. Just before this, a large amount of Ca²⁺ presumably moves across the SR membranes into the myoplasm. Therefore the early timing of the second component, with a time to peak in normal Ringer of 2–5 ms after the surface action potential, is also consistent with the speculation that the second component is signalling an SR potential change associated with the release of Ca²⁺. (The second component is similar in several ways to a fluorescence signal recently found by Bezanilla and Horowitz⁶ in whole frog muscle and also suggested by them to arise from an SR potential change).

Along these lines, a rough estimate can be made of the magnitude of this possible SR potential change, by using the signal from the surface membrane as a calibration. The calculation assumes that for a given quantity of membrane, either in the SR or on the surface, equal changes in light intensity are produced by equal changes in membrane voltage. (This assumption may be approximately correct, in that both membrane systems, considered as subcellular birefringent structures, involve cylinders of membrane extending the width of the fibre and oriented in the direction of the fibre axis.) Experiment has shown that for a range of fibre sizes the factor relating the peak size of the second component (determined in normal Ringer) to the peak size of the first component (determined in hypertonic Ringer) averages about 1.5 times the factor relating the quantity of SR membrane to the quantity of surface membrane. This latter factor has been determined for frog sartorius muscle to be about $2.7a$, where a is fibre radius measured in microns¹¹. The above assumption then implies that the magnitude of the SR potential change may be about 135 mV (1.5 times the surface action potential in 3T Ringer, which is measured to be about 90 mV).

If the ionic current causing this presumed SR potential change was primarily carried by Ca²⁺ moving from one side of the SR to the other, it is interesting to note how much increase in total myoplasmic Ca²⁺ would correspond to a 135 mV potential change. From the quantity of SR membrane found per litre of muscle¹¹ and the relationship $q = CV$, the increase is about 35 μmol of Ca²⁺ per litre of myoplasm. (For this calculation, a capacitance of $1 \mu\text{F cm}^{-2}$, a value typical of many biological membranes, has been assumed.) Because some Ca²⁺ may also cross the SR in addition to that flowing as net ionic current, 35 $\mu\text{mol l}^{-1}$ represents a minimum increase in total myoplasmic Ca²⁺. This roughly calculated quantity is therefore in reasonable agreement with the total Ca²⁺ thought to be released from the SR during a twitch, 100–150 $\mu\text{mol l}^{-1}$ (M. Endo and L. L. Costantin, personal communication). It is unclear why the peak size of the second component is a graded function of tonicity, falling by a factor of about 50 in going from normal Ringer to 3T Ringer. If the above speculation is correct, the observation is of interest since it would suggest that the amount of Ca²⁺ released during the twitch of a vertebrate fibre is a graded function of tonicity.

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letters to nature

Silicate absorption at 18 μm in two peculiar infrared sources

Two broad emission features often observed in the infrared spectra of cool stars near 10 and 18 μm are commonly attributed to thermal radiation by silicate dust grains¹. This identification is suggested by laboratory analyses of silicate minerals, which indicate strong lattice vibrations resulting from Si-O stretching modes (in the 10- μm region) and Si-O-Si bending modes (near 18 μm). For luminous sources shining through cold, intervening dust clouds which contain silicates, it is expected that both the stretching and bending bands should be observable in absorption (for sufficiently large column densities of dust). The infrared spectrum of the Galactic centre shows a deep absorption feature at 10 μm (N J Woolf, unpublished) and evidence for absorption at 18 μm (ref 2). Strong 10- μm absorption is also observed for the Orion point source 'BN' (ref 3), IRS5/W3 (ref 4), and AFCRL809-2992 (ref 5), no data demonstrating the presence of 18- μm absorption for these sources at longer wavelengths have yet appeared. We report here the detection of 18- μm absorption features in the spectra of AFCRL809-2992 and an infrared object associated with the microwave source OH 26.5+0.6 (ref 6). We attribute these features to the Si-O-Si bending mode in silicates.

The object AFCRL809-2992 is a strong infrared source found in the direction of the Cygnus X radio complex. Molecular line emission from HCN and CS has been detected by Morris *et al*⁷, the weak radio continuum flux of this source (see ref 5) indicates the absence of a compact H II region and places this object in the Class I category⁷. At 10 μm AFCRL809-2992 is apparently point-like (see ref 5). OH 26.5+0.6 is an exceptionally strong OH satellite-line emitter with a radio spectrum which is similar in appearance to that of the Type II OH/IR stars⁶. The near-infrared properties of this source, as well as spectrophotometry of the 10- μm absorption feature, will be reported elsewhere (G Neugebauer, in preparation). Our raster scans of OH 26.5+0.6 indicate that the associated infrared source is point-like at a wavelength of 20 μm .

Multifilter infrared photometry of these two objects was obtained with the 2.24-m telescope of the Mauna Kea Observatory between June and September, 1974. Neither source has an optical counterpart, so we located both by scanning the telescope for peak infrared signal. Standard beam-switching techniques were used, the focal-plane aperture was 13'' and

the beam displacement was 19'' in declination. Observations were made with intermediate bandwidth filters over the spectra range 5-33 μm . At wavelengths between 5 and 20 μm , we used stars which have been observed already^{8,9}, to establish an absolute flux calibration. At 25 μm and 33 μm we normalised to α Boo and α Ori, respectively, the calibration at 33 μm has been discussed in greater detail elsewhere¹⁰.

Our observations are summarised in Fig 1 along with published spectrophotometry of the AFCRL source⁵. The observational uncertainties in the photometry are estimated to be 10% or smaller. Clearly, the spectra of both objects show pronounced absorption features near 10 and 18 μm . Our photometry of the OH source does not delineate sufficiently the 10- μm band to show the deepest (central) part, the observations of the AFCRL source indicate, however, that maximum absorption occurs near 9.8 μm . Similarly, we cannot locate precisely the position of maximum absorption at longer

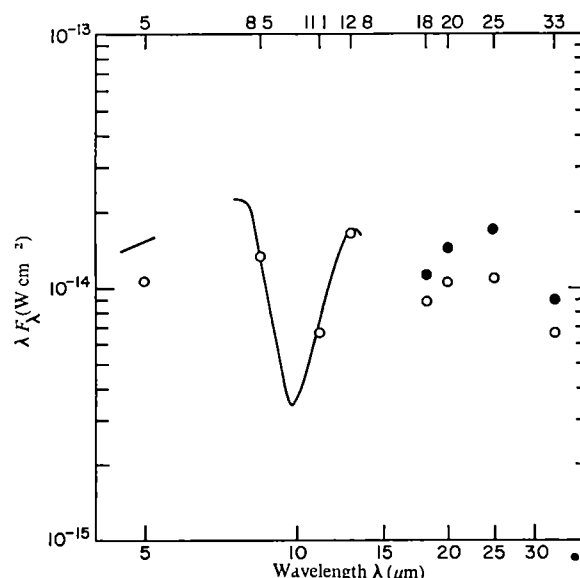


Fig 1 Observations of two peculiar infrared sources. O, Photometry of OH 26.5+0.6, ●, photometry of AFCRL809-2992, —, spectrophotometry of the AFCRL source from ref 5.

wavelengths, but we infer from the spectrophotometry of the AFCRL source that band centre must lie between 16 and 18 μm (If the position of the band centre actually falls short of about 16.1 μm , then it will be unobservable from the ground because of absorption by the Earth's atmosphere.) The observed positions of the absorption features are in reasonable agreement with the computations of Knacke and Thomson¹¹, which predict that the Si-O stretching band falls between 9.1 and 9.5 μm and that the Si-O-Si bending band lies between 16.7 and 18.5 μm .

An estimate of the optical depth of the 18- μm absorption band of the AFCRL source can be obtained from a model similar to that discussed by Merrill and Soifer⁵, that is, an emitting source, with a spectrum which follows a Planck curve of $T \sim 350$ K, is assumed to lie behind a cold, absorbing dust cloud. If the Planck curve is normalised to the observed fluxes at 5 and 33 μm , at which wavelengths the opacity of the overlying dust is reduced substantially from the peak near 10 μm , then the optical depth of the cloud at 18 μm is ~ 1.0 . The optical depth at 10 μm for this model is approximately 2.6, giving the ratio $\tau_{18 \mu\text{m}}/\tau_{10 \mu\text{m}} \approx 0.38$. Although the derived optical thickness of the cloud depends on the source spectrum adopted, the opacity ratio is somewhat less sensitive to this assumption. For example, if a smooth curve is drawn through the flux peaks at 5, 8 and 25 μm to represent the intrinsic energy distribution of the emitting source, the ratio $\tau_{18 \mu\text{m}}/\tau_{10 \mu\text{m}} \approx 0.35$ can be derived. The calculations of extinction cross sections for lunar silicates¹¹ yield the range 0.35–0.55 for the extinction ratio, which is in good agreement with the estimates discussed. We conclude, therefore, that the present observations of the position of the 18 μm absorption, and its strength relative to the 10- μm absorption, provide strong evidence to support suggestions that the absorbing agent is silicate dust.

The infrared flux received at the Earth from the AFCRL source at wavelengths between 2 and 33 μm is $3.3 \times 10^{-14} \text{ W cm}^{-2}$, approximately 20% of that energy falls within the 20–33- μm band. If the source is located at a minimum distance of 2 kpc (ref 5), as suggested by the molecular line emission at radio wavelengths, then the bolometric luminosity of the object exceeds $4.0 \times 10^4 L_{\odot}$. Similarly, the flux measured for OH 26.5+0.6 between 5 and 33 μm is $2.1 \times 10^{-14} \text{ W cm}^{-2}$. The distance to that source is unknown, however, assuming a nominal value of 500 pc (the distance to the Orion Nebula) a luminosity greater than $1.6 \times 10^3 L_{\odot}$ is obtained. For comparison, the total luminosity of the Orion point source is estimated to be $1.5 \times 10^3 L_{\odot}$ (ref 12).

By considering the observed infrared flux and distance of the Orion source, Becklin *et al.*¹² have concluded that BN cannot be simply a normal, luminous star hidden behind a dense interstellar dust cloud. The strong infrared signals and spectral similarity to BN of the two objects studied here imply a similar conclusion for AFCRL809–2992 and OH 26.5+0.6. Observations at higher resolution from 1 to 33 μm would undoubtedly be of importance in clarifying the relationship of these sources to objects such as BN and IRS5/W3, both of which are likely to be protostars still enshrouded by remnant dust shells.

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Recent increase in Jupiter's decimetric radio emission

DURING an observational programme in support of a study of the linear polarisation properties of Jupiter's decimetric radio emission, it was noted that the planet's total intensity had increased between late summer 1973 and July 1974. The increase is small ($\sim 5\%$) but it seems to be a significant change in the trend of the past decade, during which time Jupiter's decimetric emission has decreased by 20–30% (refs 1–3) with respect to the flux densities observed before 1967.

The measurements were made at Goldstone, California, with the NASA 64-m parabolic antenna operating at 13.1 cm wavelength (2,295 MHz). Jupiter was observed on four nights between July 16 and September 11, 1973, and again on four nights between July 3 and August 17, 1974. The receiving system consisted of a rotatable waveguide polariser, a low-noise travelling-wave maser, and a noise-adding radiometer⁴ that provided stabilisation against short term gain variations. The linear polarisation parameters were determined from consecutive off-on-off measurements of Jupiter made with the polariser set to four different position-angle values at intervals of 45° . Antenna pointing errors were measured and corrected at regular intervals and the system gain was calibrated throughout each night by observing at least three of the radio sources 3C17, 3C48, 3C138, 3C286 and NGC7027. The flux densities of the calibration sources were subsequently determined relative to the radio galaxy Virgo A which has a flux density⁵ of 140.3 Jy at 2,295 MHz.

Jupiter's total intensity was computed for each set of four off-on-off measurements. The data are plotted as a function of the longitude of the central meridian (System III 1957.0) in Fig. 1. Both data sets exhibit the familiar periodic intensity variation with longitude (the beaming curve) but the 1974 data points are consistently above the corresponding 1973 values. The offset is several times greater

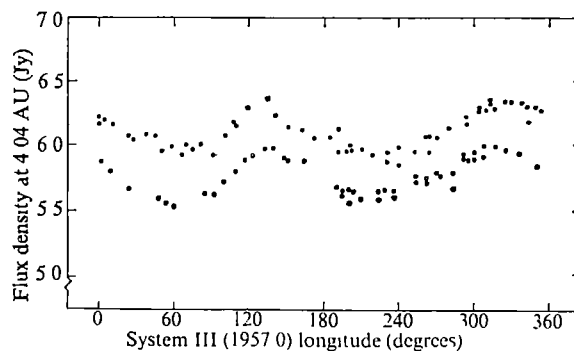


Fig. 1 The flux density of Jupiter at 13.1 cm is plotted as a function of System III (1957.0) longitude. O, data taken in 1973; ●, data taken in 1974.

than the uncertainty in the measurement caused by calibration discrepancies or by the effects of background radio sources near Jupiter's position in the sky. So I attribute the difference in flux density to an increase in Jupiter's decimetric radio emission.

It would be incorrect to measure the increase in the decimetric emission simply by determining the offset of one complete beaming curve with respect to the other because the shape of the observed curves varies as a function of D_E , the Jovicentric declination of the Earth⁵. But the strengths of the intensity peaks, which occur twice each rotation when the line of sight from Earth lies in Jupiter's magnetic equatorial plane, are expected to be independent of D_E . The flux densities near these two peaks were determined by fitting second order polynomials to the data with longitudes within $\pm 30^\circ$ of the peaks. The maximum flux densities in 1973 are 5.98 Jy and 5.99 Jy for the first and second peaks shown in Fig. 1. The corresponding values for 1974 are 6.35 Jy and 6.34 Jy. The average increase is 0.36 ± 0.06 Jy, the quoted uncertainty is chiefly the result of uncertainty in the relative calibration of the system from 1973 to 1974.

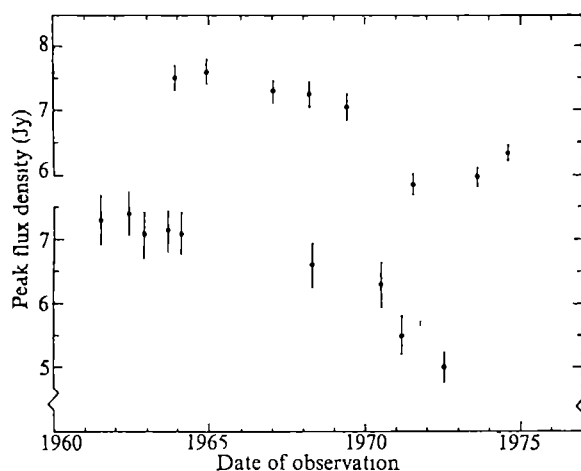


Fig. 2 Jupiter's peak flux density, normalised to a distance of 4.04 AU, is plotted against the date of the measurement. ●, Measurements at 11.1 cm (refs 7-9), 12.6 cm (ref. 1) and 13.1 cm (ref. 6), ○, a collection of measurements at 21 cm as assembled and published by Berge (ref. 3).

In Fig. 2 the average peak flux densities measured at wavelengths of 21 cm and 11-13 cm are plotted as a function of the epoch of each observation. The error bars, which range between 2 and 5%, are estimates of the relative standard errors. The flux density in both wavelength intervals seems to have decreased monotonically until mid-1972. The two 13-cm measurements reported here are the first to show a significant reversal of this downward trend and they demonstrate that Jupiter's decimetric flux density is variable with time scales as short as one or two years.

It is generally believed that the variations are related to changes in the properties of the Jovian Van Allen radiation belts, but specific causes of the variability have not been determined. Attempts to identify periodic variations that might be related to the 11-yr cycle of solar activity or the 12-yr period of the Jovian year have been unsuccessful¹⁻³. Reports of pronounced intensity variations occurring within weeks or months have been published. The most recent of these reports⁷ described variations as great as 30% occurring within a few days in 1968. In contrast, several high precision measurements made with the CSIRO 64-m antenna indicated that the Jovian flux at 11 cm and 21 cm was constant during the period 1963-67 (refs 8-10), and observations at 13 cm showed no variations larger than 2% during a 6-month interval in 1971 (ref. 1).

Variations in the intensity of Jupiter's radio emission is relevant to the analysis of particle and field data returned from spacecraft such as Pioneer 10 and 11. If the intensity variations are caused by physical changes within the Jovian radiation belts, the environment measured by Pioneer 11 will probably be different from that measured by Pioneer 10.

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Shape of Mars

THE geometrical ellipticity of Mars differs considerably from the dynamical ellipticity obtained from the precession of the orbits of the satellites. Study¹ of the Martian centre of mass lead Schubert and Lingenfelter to comment that the location of the Hellas basin near the direction of the thinnest crust, as implied by the offset, might suggest that an impact produced the asymmetric crustal distribution. If such an impact did occur then it is likely to have also affected the shape of Mars. An order of magnitude estimate can therefore be made, from the shape of Mars, of the required size and velocity of the impacting body.

I take the value for the geometrical ellipticity e_s to be 0.0089 ± 0.0015 (ref. 2). For the dynamical ellipticity e , which is related to the second harmonic of the planets gravitational field, I take the value 0.005238 ± 0.00009 (ref. 3). Assume now that the rotation of Mars was greater at some past epoch for which the whole planet was in hydrostatic equilibrium. For such a case the ellipticity e_h can be defined as the fractional excess of the equatorial radius over the polar radius. The Darwin-Radau formula gives

$$e_h = \delta \left[\frac{2}{5} + \frac{5}{2} \left(1 - \frac{3}{2} \frac{C}{Ma^2} \right) \right] \quad (1)$$

where δ is the ratio of the centrifugal to the gravitational acceleration at the equator, C is the moment of inertia about the axis of rotation, M is the mass and a is the mean radius of Mars. I now assume that as a first approximation the ratio C/Ma^2 and the ratio a^2/M may be treated as remaining constant. Then

$$e_h = k_1 \delta = k_2 \Omega^2 \quad (2)$$

where k_1 , k_2 are constants of proportionality and Ω is the angular rotation of Mars. The assumption of total hydrostatic equilibrium gives the relationship

$$e = e_s = e_h \quad (3)$$

Let Ω_1 be the angular rotation of Mars before impact and Ω_2 its present angular rotation, for simplicity consider the axis of

rotation as unchanged I now assume that most of the planet is in hydrostatic equilibrium but that the surface has approximately the old ellipticity. Thus to obtain the value of Ω_1 which will make $e_h = 0.0089$, $\Omega_1 \approx 1.3\Omega_2$ (from equation (2))

To obtain some idea of the size and velocity which an impacting body may have to have in order to achieve this reduction in the angular velocity I consider a simple two dimensional situation where a body of mass m impacts with a velocity v tangentially to the surface of Mars and along its equator. I assume that the direction of impact is such as to reduce the rotation of Mars.

In an impact of this size dispersion of material would be expected to result and in a more exact treatment one would have to integrate the angular momentum about the axis of rotation of Mars for a distribution of secondary impacting bodies as well as for the primary object. Instead I assume a non-elastic rigid body impact which makes our treatment an approximate one. If the moment of inertia of Mars (about its axis of rotation) is $0.376Ma^2$ then from conservation of angular momentum

$$\begin{aligned}mv &\approx 0.376Ma(\Omega_1 - \Omega_2) \\ &\approx 0.1Ma\Omega_2 \\ v &\approx 20(M/m)\end{aligned}\quad (1)$$

since $a\Omega_2 \approx 200 \text{ m s}^{-1}$

The orbits of the asteroids are very varied, some have eccentricities of 0.8 or greater. Some asteroids have orbits which take them past the orbit of Jupiter. I now select an orbit which will give a large impacting speed and which is compatible with existing asteroid orbits to see whether this will give a reasonable mass for the impacting body. The orbit chosen is one which has an ellipticity of 0.8 and which comes close to the orbit of Jupiter. For simplicity take the orbit of Mars as a circle with unit radius. Take the elliptical orbit to be $r_{\max} = 3$ and $r_{\min} = \frac{1}{3}$ where r_{\max} and r_{\min} are the aphelion and perihelion distances. This gives a value of 0.8 for the ellipticity e , and r_{\max} is just less than the mean radial distance of Jupiter.

If I now take the Sun as origin I have for the equations of the orbits of Mars and of the impacting body

$$x^2 + y^2 = 1, \quad (x - c)^2 + y^2 / (1 - e^2) = a^2$$

where $a = 5/3$, $e = 4/5$ and $c = ae = 4/3$. For such an orbit (on calculations made using standard two-dimensional orbit theory) the speed of the impacting body at the moment of impact is $\sim 30 \text{ km s}^{-1}$ at an angle of $\sim 19^\circ$ to the major axis of the elliptical orbit. The speed of Mars is $\sim 24 \text{ km s}^{-1}$ at an angle of $\sim 150^\circ$ to the major axis of the elliptical orbit. So the impact speed relative to Mars is $\sim 32 \text{ km s}^{-1}$. This value could be increased slightly if the acceleration due to gravitational attraction was to be taken into account. Substituting this value for v in equation (1) gives as the required mass of the impacting body the value $m \approx 0.7 \times 10^{-3}M$. If the impact is assumed to be tangential at latitude 45° (approximately that of the Hellas basin) then $m \approx 10^{-3}M$ but if it is at an angle of 45° to the vertical then $m \approx 1.4 \times 10^{-3}M$. These values for m are about the same as the mass of the largest asteroid, assuming that the densities of Mars and of the asteroid are the same.

These calculations were made on the assumption that the difference between the geometrical and dynamical ellipticities is due to collision only. If other factors such as convection⁴ play a role the required size of the asteroid and the value of the orbital eccentricity of the impacting body may even be smaller.

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CO₂ and HCN in sunspots

THE triatomic molecules CO₂ and HCN have now been reported to be present in cool stars^{1,2}, and H₂O had already been detected in the sunspot spectrum³⁻⁷. Dissociation equilibrium calculations for three recent sunspot models⁸⁻¹⁰ show that the molecules CO₂ and HCN are fairly abundant in sunspots. The results for the Zwaan model have already been published¹¹, we further find that the abundances of CO₂ and HCN are comparable for all three models. We have therefore calculated equivalent widths of selected lines of these molecules to ascertain if they might show up in the infrared region of the sunspot spectrum. The strongest fundamental bands of HCN and CO₂ and a weaker band of CO₂ were included in our calculations. The selected J values correspond closely to the expected maximum Local Thermodynamic Equilibrium (LTE) population under sunspot conditions.

The sunspot model selected for the equivalent width calculations is that of Stellmacher and Wiehr¹⁰. The procedure for calculating the equivalent widths has been outlined earlier¹². Two R (56) lines of CO₂ at wavenumbers $3,478.93 \text{ cm}^{-1}$ and $2,384.49 \text{ cm}^{-1}$, belonging respectively to the bands (10^01-00^00) and (00^01-00^00) of the $\Sigma-\Sigma$ transitions, were selected. The equivalent width of the former line at the centre of the disk turned out to be 0.032 mÅ suggesting an absence of the (10^01-00^00) band in the sunspot spectrum. Details of the centre-to-limb variation of equivalent width for the (00^01-00^00) line are given in Table 1.

Table 1 Equivalent width of (00^01-00^00) line

$\cos\theta$	1	0.7	0.5	0.3
Equivalent width (mÅ)	6.6	7.7	7.8	11.3

We are not aware of any observations of the (00^01-00^00) band of CO₂ in the $4.3 \mu\text{m}$ region of the sunspot spectrum and therefore a verification of these predictions with observations has yet to be done.

The R (25) line of HCN at $3,380.84 \text{ cm}^{-1}$, belonging to the band (00^01-00^00) of the $\Sigma-\Sigma$ type transition, in the Stellmacher-Wiehr¹⁰ sunspot model has an equivalent width of 0.072 mÅ at the centre of the disk. The fact that the other fundamental bands at 712 cm^{-1} and $2,089 \text{ cm}^{-1}$ have lesser integrated intensities¹³ leads us to suggest that the HCN fundamental bands may not show up in the infrared region of the sunspot spectrum.

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Re-interpretation of Devensian Till stratigraphy of eastern England

THE current reconstruction of the Devensian Till stratigraphy of Holderness¹ invokes three units—the Drab, Purple and Hesse Tills—with the Hesse overlapping both lower tills so as to be the only one present in the westernmost part of the area, on the Yorkshire Wolds. Because of the characteristic reddish brown colours (usually 5YR4/4 of the Munsell Soil Colour Charts, 1954) of the uppermost tills throughout eastern England, the upper till in Lincolnshire and the Hunstanton Till of north Norfolk are equated usually with the Hesse Till¹⁻⁵, although Straw⁶ correlated his two Marsh Tills in Lincolnshire with the Drab and Purple Tills of Holderness, and Bisat⁷ equated the Hesse Till of the Yorkshire Wolds with his Middle Drab division.

The results of a mineralogical study on these tills⁸ have shed new light on these stratigraphic correlations. In Yorkshire, the Drab and Purple Tills are found to be essentially uniform in both texture and mineralogy, but distinct from one another, whereas the Hesse Till undergoes a transition in these properties between south-eastern Holderness and the Wolds. Maps (Fig 1a) may be drawn for various textural and mineralogical parameters, on the basis of which three zones can be distinguished (Fig 1b). Zone C covers western and northern parts of Holderness and the Yorkshire Wolds. No mineralogical or textural distinctions could be drawn between Straw's⁶ Upper and Lower Marsh Tills, and both these and the Hunstanton Till resemble closely the Hesse Till of Zone C in Holderness. This till is also texturally and mineralogically similar to the Drab Till, on which it can be seen to lie in deep sections. The only significant differ-

ences are the colours of the two tills, and the almost complete absence of siderite and pyrite from the coarse silt (20–53 μm) and fine sand (53–250 μm) fractions of the Hesse Till. The Hesse Till of Zones A and B likewise resembles the Purple Till in all but these respects. The use of selected mineral ratios (Fig 2) brings out most clearly the relationships between the various tills.

The coarse silt mineralogy of the Hesse Till of Zone C resembles that of loessial material that overlies the chalk on the Yorkshire and Lincolnshire Wolds and is also incorporated in a chalky head deposit beneath the Hesse Till⁹. Incorporation of loess in the till of western Holderness and the Wolds could thus be invoked to account for the change in composition of the Hesse Till. A large admixture of silt would however, be necessary but the Hesse Till of Zone C contains less silt than that of Zones A and B. Also, there are parallel changes in the fine sand mineralogy that could not be explained in this way, as loess contains very little fine sand.

The variability of the Hesse Till across Holderness may be related to the direction of ice movement, as the isopleths (Fig 1a) are approximately parallel to Devensian ice fronts postulated by several authors¹⁰. Similar variations occur in Wisconsinan tills in Ohio and Pennsylvania and are ascribed to progressive incorporation of underlying bedrock of a mechanical and mineralogical composition different from that of the original till material¹¹. There, however, the changes were greatest in the lowest till, whereas in Holderness it is the uppermost till that is most variable. Thus, the 'contamination hypothesis' only seems tenable if the Hesse Till was the product of a separate ice advance. Catt and Penny¹ could find no evidence to support that

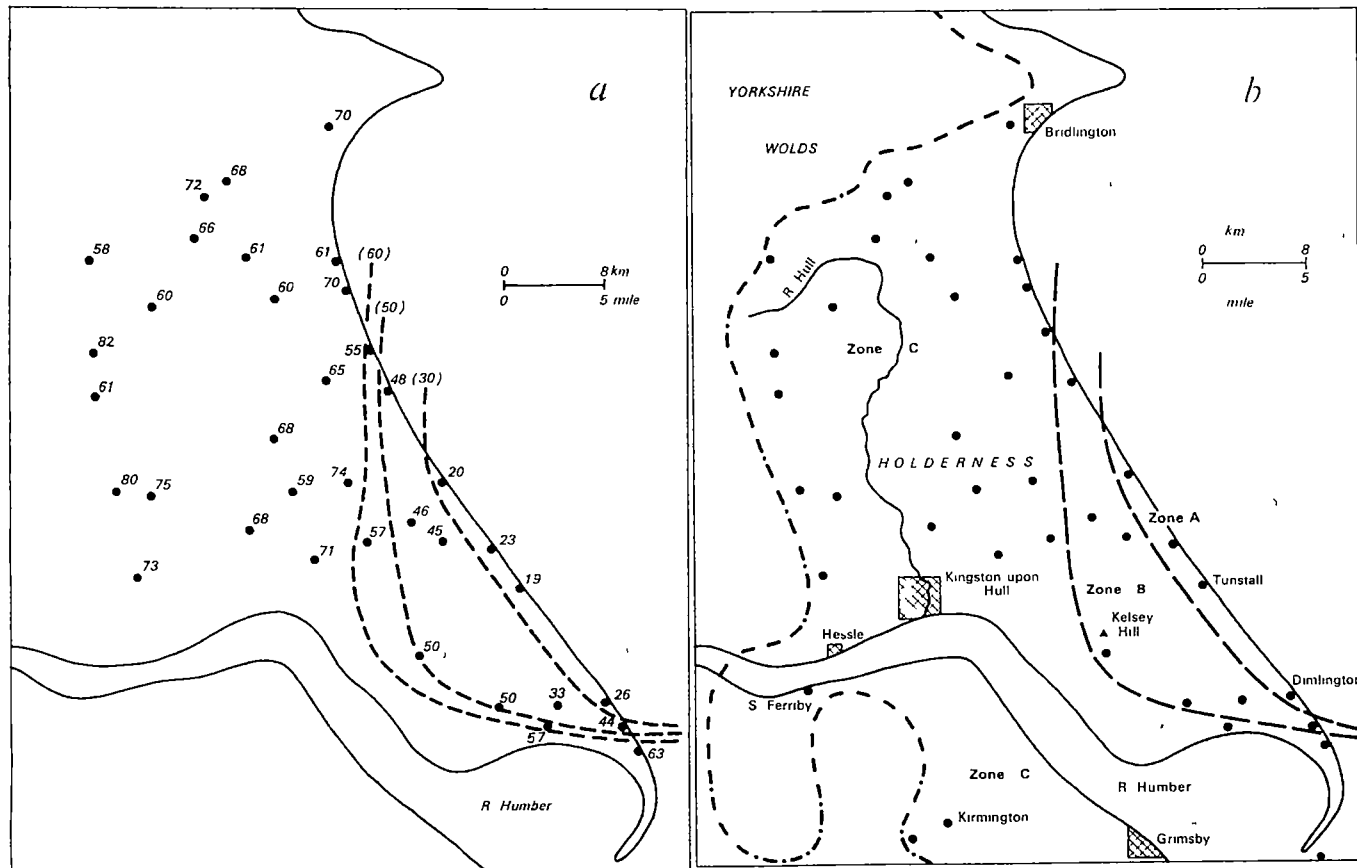


Fig 1 a, Mineralogical variability of the Hesse Till, 53–250 μm fraction (amphibole/(amphibole+pyroxene) $\times 100$), b, zonation of the Hesse Till in Holderness. ●, sampling sites, ---, western limit of Devensian tills

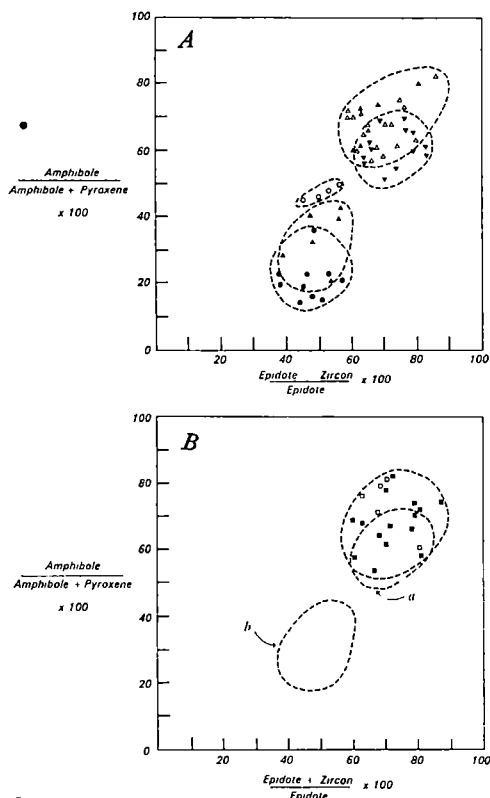


Fig. 2 Relationships between tills shown by mineral ratios
 A ●, Hessle Till, Zone A, ○, Hessle Till, Zone B, △, Hessle Till, Zone C, ▲, Purple Till, ▼, Drab Till B ■, Marsh Till, □, Hunstanton Till, a, Drab Till, b, Purple Till

suggestion, and regarded the entire Drab–Purple–Hessle sequence as the product of a single, composite Devensian ice sheet

Most of the variation in the Hessle Till seems to be related to the extent of the Purple Till, which is seen only in the cliffs of south-eastern Holderness and probably thins out westwards from the coast, as it is absent in the Hull Valley. The Hessle Till of Zone A is always underlain by Purple Till, and is mineralogically and texturally similar to it, whereas that of Zone C overlies Drab Till or Chalk, and is mineralogically and texturally similar to the Drab Till. The Hessle Till of Zone B occupies an intermediate position,

geographically, mineralogically and texturally, and may coincide with the feather edge of the Purple Till sheet

The exact junction between the Hessle Till and the underlying Drab Till is difficult to place, because the colour change often extends vertically downwards along fissures in the underlying grey (Drab) till, giving rise to reddish brown partings within it. Colour boundaries such as that indicate oxidative weathering, and siderite and pyrite, which are common in the Drab and Purple tills, but virtually absent in the Hessle, Marsh and Hunstanton tills, are both oxidised easily to hydrated ferric oxides, which would give the oxidised till a reddish brown colour. The grey Lower Marsh Till at South Ferriby (SE 996223) in Lincolnshire contains both siderite and pyrite, whereas the red till above it, which resembles the Hessle Till, lacks both minerals but is otherwise texturally and mineralogically identical to the grey till. Samples collected at metre intervals through the Hessle Till into the Purple Till at Dimlington (TA 398207) also proved to be mineralogically similar except for siderite and pyrite, both of which were present in samples close to and below the colour change taken as the boundary between the two tills. Further samples across the boundary between Purple and Drab tills at Dimlington High Land (TA 393215) and Aldbrough (TA 259395) showed that a mineralogical gradation occurs over a few metres close to this junction.

That leads to the conclusion that the Hessle Till, as currently defined, is in reality a composite unit. The reddish brown till of Zone C is the weathered upper part or feather edge of the Drab Till, and that of Zone B is probably the weathered remains of the thin westward extension of the Purple Till. That of Zone A is the weathered upper layer of the Purple Till. The name 'Hessle Till' is thus misleading stratigraphically, at the type locality it is, under the new interpretation, weathered Drab Till, whereas in south-eastern Holderness it is weathered Purple Till. Therefore, I propose that usage of the term be discontinued.

Straw⁶ subdivided the Marsh Tills of Lincolnshire into Upper and Lower units on the basis of their position either side of a 're-advance limit' of the last glaciation, which he recognised geomorphologically. I could detect no lithological differences between the two divisions, both tills were similar to the 'Hessle Till' of Zone C in Yorkshire. As this is now recognised to be weathered Drab Till, it seems reasonable to suggest that the Marsh Tills are also Drab or weathered Drab. A similar argument applies to the Hunstanton Till, which seems to be the oxidised, southernmost extension of the Drab Till. No till resembling either the

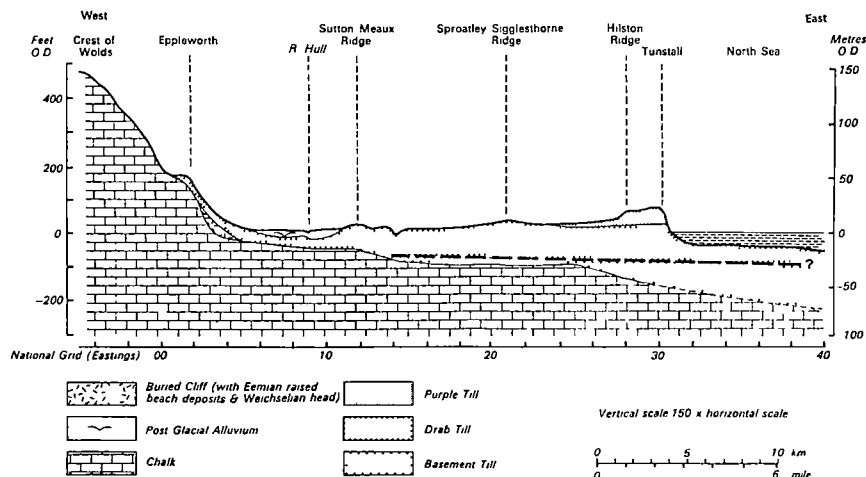


Fig. 3 East-west section across Holderness

Purple or the 'Hessle' of Zone A occurs in Lincolnshire or northern Norfolk

Many workers^{1,4-6,10} have cited the fresh, relatively undissected glacial morphology of Holderness, eastern Lincolnshire, and the coastal strip of northern Norfolk, in arguing for a Devensian age for the tills. This suggests the present distribution of the Drab and Purple Tills is depositional, and not primarily a result of subsequent erosion. The stratigraphic relationship of the Devensian Tills of eastern England is therefore one of offlap (Fig. 3) and not of overlap¹

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Copper in surface waters south of New Zealand

THE sampling of the transition metals, including copper, in the ocean has so far produced unsatisfactory results. Reported measurements show quite a large scatter within a given suite of data and significant differences between investigators¹⁻³. Published profiles in general look quite unlike any of the 'accepted' distributions for other dissolved species outlined later nor are there any close similarities between the transition elements themselves. Either they have a unique geochemistry or the data are to some degree invalid. For copper, contamination during sampling has been suggested⁴ as one source of error, and we

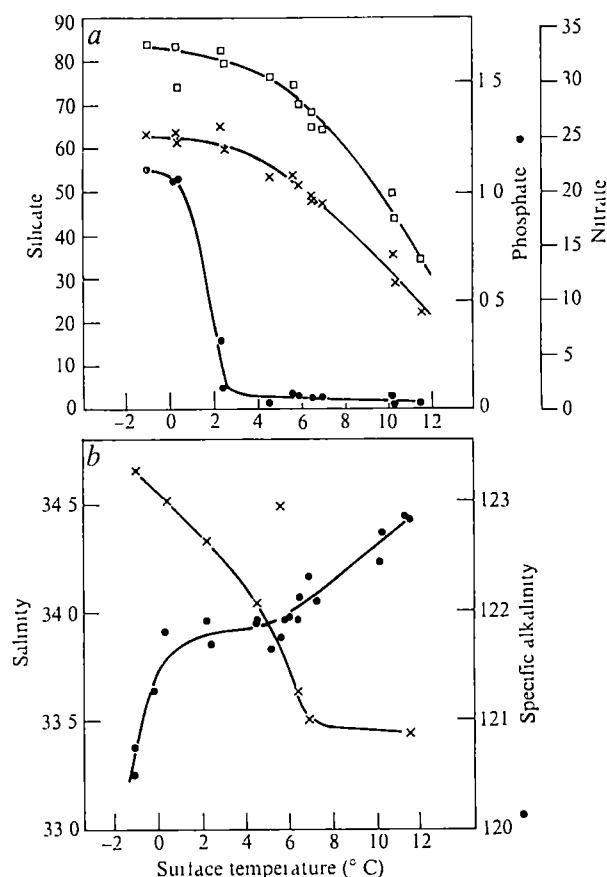


Fig. 1 a, Plots of surface values for phosphate, nitrate and silicate for all stations on GEOSECS Leg G against surface temperature. Units are $\mu\text{mol kg}^{-1}$. □, Phosphate, ×, nitrate, ●, silicate. b, Plots of specific alkalinity and salinity against surface temperature. For specific alkalinity (alkalinity/chlorinity) the unit is $\mu\text{eq per g chloride}$. ×, specific alkalinity, ●, salinity.

have tried to overcome this by sampling surface waters in regions of marked horizontal gradients in chemical properties.

Present understanding of the controls on the distribution of dissolved species in the ocean has been derived largely from inferences based on the form of the distributions themselves. Five types are presently recognised:

- (1) constant relative composition (Na, K, Mg, Cl, SO_4^{2-}) as a result of very low reactivity in the ocean environment,
- (2) surface depletion and deep water enrichment (P, N, C, Sr,

Table 1 Preliminary station data for surface waters on Leg G of the Pacific GEOSECS expedition

Station	Location	T (°C)	S (‰)	PO ₄ ($\mu\text{mol kg}^{-1}$)	NO ₃ ($\mu\text{mol kg}^{-1}$)	Si ($\mu\text{mol kg}^{-1}$)	A _{sp} ($\mu\text{eq g}^{-1}$)	Cu (nmol kg ⁻¹)
280	56°1'S, 170°3'E	6.45	34.061	1.30	19.2	2.9	121.3	2.27
282	57°35'S, 169°36'E	5.58	33.880	1.49	21.5	3.4	123.0	2.57
				1.55*	22.4	0.9		
284	59°31'S, 170°0'E	5.85	33.957	1.41	20.6	3.2	—	1.80
285	61°29'S, 169°58'E	2.24	33.963	1.65	25.9	15.9	122.7	2.52
				1.67*	26.6	15.6		
286	66°5'S, 173°40'E	0.30	33.907	1.48	24.5	52.7	123.0	3.05
				1.55*	25.2	53.8		
287	69°5'S, 173°30'W	-1.07	33.377	1.68	25.2	54.9	123.3	3.25
				1.59*	25.9	56.6		
290	58°0'S, 174°0'W	4.53	33.958	1.53	21.4	1.4	122.1	1.87
				1.52*	22.0	3.7		
292	54°5'S, 176°58'W	10.25	34.358	0.88	11.5	1.4	—	0.98
293	52°40'S, 178°5'W	11.47	34.426	0.69	9.0	1.8	120.9	1.33
294	50°38'S, 179°59'W	10.16	34.230	0.99	14.2	3.0	—	1.28

* Where the surface sample was collected more than 2 h before or after the shallow hydro cast, nutrients were also determined on the trace element water sample on the ship. The values are given below the station values.

- IO_3^- , Si, Ca, alkalinity, Ba, ^{226}Ra)^{5,6} caused by incorporation in the biogeochemical cycle (dissolved oxygen is a special 'inverse' case involved in the same cycle),
- (3) mid-depth and near-bottom depletion (^{210}Pb , ^{210}Po) caused by adsorption on suspended particles,
 - (4) pronounced mid-depth maximum (^3He) caused by injection at spreading ridge crests,
 - (5) near-bottom enrichment (^{222}Rn , ^{226}Ra , ^{228}Ra) caused by diffusion of radiogenic isotopes out of sediments

To date no categorisation has been made for any of the transition metals. Patterson⁴ has suggested that in spite of increasingly stringent precautions taken by trace element geochemists, a major unsolved problem is contamination during sampling. This problem can be most easily circumvented by restricting sampling to surface waters, however, the major criterion for validity of data is that it be oceanographically consistent. To apply this constraint requires sampling in regions of marked horizontal gradients in chemical properties. Such an opportunity arose on Leg G of the Pacific GEOSECS expedition, which made two sections south of New Zealand across the Circumpolar Current to $\sim 75^\circ\text{S}$. In this region major upwelling of deep water takes place, intensifying southwards. Plots of cruise data against surface temperature (Fig. 1) or latitude show

systematic increases for phosphate, nitrate and specific alkalinity and a step-like distribution for silicate.

Samples were collected on station from the bow of the ship. The sampler was a 4-l polyethylene jerry jug which had been leached with 0.1 N hydrochloric acid for 24 h, rinsed four times with very pure distilled water, and stored in a polyethylene bag. The jug was supported in a harness of polypropylene line. A metal weight encased in plastic was suspended about 6 m below this with similar line. First, the capped jug was lowered into the water and the exterior rinsed. It was then retrieved, the cap removed, resubmerged, and allowed to fill. The jug filled at a depth of at least 3 m. On recovery the jug was capped and within 5 min acidified with about 20 ml of 6 N hydrochloric acid which had been redistilled from a Vycor still. The bottles were stored in sealed polybags until analysis 2 months later.

Copper was preconcentrated from the samples by coprecipitation with cobalt pyrrolidine dithiocarbamate. The precipitate was redissolved in a small volume of methylethyl ketone with 5% 0.1 N HNO_3 . Copper was then determined by flame atomic absorption; the estimated precision of the analysis is $\pm 0.15 \text{ nmol kg}^{-1}$ and the blank is $0.45 \text{ nmol kg}^{-1}$.

The results of the analysis are given in Table 1 together with the relevant hydrographic and station data. The copper concentrations are all near the low end of the range of recently reported values^{1,2} ($1\text{--}32 \text{ nmol kg}^{-1}$) and exhibit variations of almost a factor of three that are correlated with surface temperature. In Fig. 2 the data are plotted against nitrate. This is the most precisely determined tracer of upwelling, the analytical precision being about 0.6% (for phosphate 0.7%). Also shown are similar plots for phosphate, silicate, salinity and specific alkalinity (A_{sp}). It is apparent from this and from Fig. 1 that the distribution of silica is quite distinct and that the copper-nitrate correlation resembles closely that for phosphate and alkalinity. The inverse relation for salinity reflects the precipitation patterns in the region: the existence of the Antarctic Circumpolar Front, at $\approx 4^\circ\text{C}$ precludes a simple mixing relationship.

The linear copper-nitrate trend has a least-squares standard error of 0.3 nmol kg^{-1} and the line passes through the origin ($-0.07 \text{ nmol kg}^{-1}$ at zero nitrate). The correlation coefficient is 0.88 and the molar ratio Cu/N is 1.09×10^{-4} giving a 'Redfield ratio' Cu/P of 1.7×10^{-3} . The recently reported average in plankton ash⁷ is 6.6×10^{-3} . Although the covariance of copper with the nutrients phosphate and nitrate is plausible, given the biochemical importance of copper⁸, it is not uniquely established from these data.

The scatter in the alkalinity data makes it difficult to relate to the other nutrients: a covariance of copper and alkalinity would, however, require that biogenic carbonates contain approximately 60 p.p.m. Cu. Arrhenius⁹ reported 25 p.p.m. in foraminiferal ash with 15 p.p.m. being associated with the carbonate fraction. Aragonitic corals generally show values less than 10 p.p.m. (ref. 10). *Acantharia radiolaria* which precipitate shells of celestite (SrSO_4) have been reported as containing 750 p.p.m. Cu (ref. 9). Accepting the general correlation of strontium with phosphate in the water column the average Sr/P ratio of 0.67 would require 840 p.p.m. Cu in the strontium carrier phase. Extrapolation of these hypothetical copper-carbonate and copper-strontium relationships gives zero copper at a specific alkalinity value of 119 and a strontium concentration of $87 \text{ } \mu\text{mol kg}^{-1}$, that is, copper is exhausted long before the major carrier components: this is highly unusual behaviour for a solid solution.

In conclusion, it is definitely established that relative to lower latitudes, copper is enriched in the surface waters of the Antarctic upwelling areas, and, by extension, in the deep waters of the ocean. If the correlation with nitrate is valid as described here, then copper can be classified as a limiting nutrient. The levels in the surface water in low and mid-latitudes should be very close to zero (less than 0.1 nmol kg^{-1}) and the maximum values in the deep Pacific should not exceed 5 nmol kg^{-1} .

We thank the officers, crew and scientific party of RV Melville for their help and the members of the Science Advisory

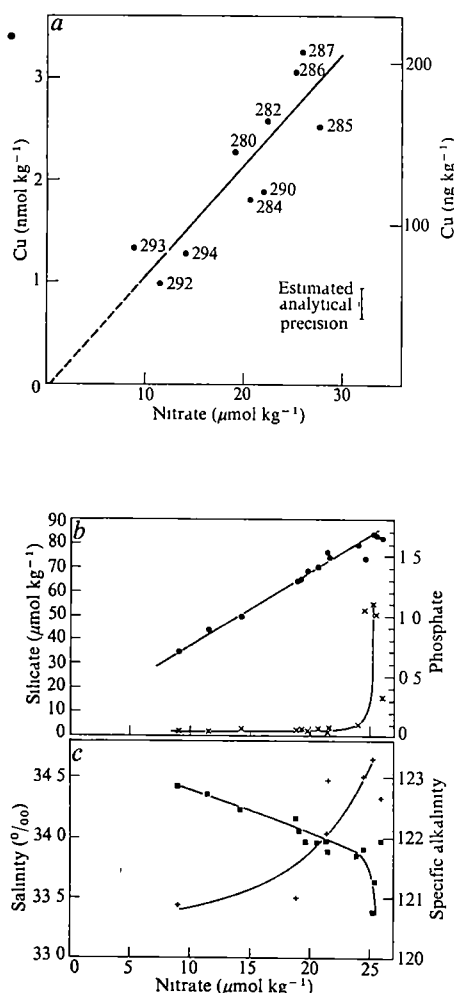


Fig. 2 a, Plot of the copper data against nitrate: station numbers are given adjacent to the points. b, Plots of surface phosphate and silicate against nitrate for all stations on Leg G. ●, Phosphate, ×, silicate. c, Plots of specific alkalinity and salinity against nitrate. ■, salinity, +, specific alkalinity.

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Drag reduction by collapsed and extended polyelectrolytes

I REPORT experiments on drag reduction by saline solutions of a partially hydrolysed polyacrylamide (PAMH) showing the effect of macromolecule conformation. The extreme collapsed and extended PAMH conformations also simulate the deportment of linear random-coiling macromolecules in solution, and of high aspect ratio fibres in suspension, and they indicate a possible relationship between the mechanisms of drag reduction produced by these additives.

A commercial PAMH polymer, B1110 (Betz Laboratories), $M = 15 \times 10^6$ was used. This has an intrinsic viscosity, $[\eta] = (2,300, 2,550, 6,000, 16,000, 52,000) \text{ cm}^3 \text{ g}^{-1}$ in (1.0, 0.1, 0.01, 0.001, 0.0) M NaCl solutions respectively. The radius of gyration of B1110 in 1.0 M NaCl was 315 nm, in dilute distilled water solution B1110 is expected to be greatly extended, to an end-to-end distance of the order of its contour length, $\sim 70 \mu\text{m}$.

Friction results for collapsed and extended conformations are shown in Fig 1, using Prandtl-Karman coordinates with abscissae based on solvent viscosity. The behaviour of solutions of collapsed polyelectrolyte, Fig 1a, is identical with that established¹ for random-coiling macromolecules. Solutions of 1 and 10 parts per million (by weight) of B1110 follow curve (1) for $Re_s f^{1/2} < 200$, transit to curve (2), exhibit an onset of drag reduction at $Re_s f^{1/2*} \sim 400$ and follow straight lines for $Re_s f^{1/2} > Re_s f^{1/2*}$ with slopes exceeding that appropriate for a Newtonian fluid. B1110, 100 p.p.m., follows curve (1), $Re_s f^{1/2} < 250$, and curve (3), $Re_s f^{1/2} > 350$. Among solutions of extended polyelectrolyte, Fig 1b, 10 p.p.m. B1110 in distilled water follows curve (1) for $50 < Re_s f^{1/2} < 200$, for $200 < Re_s f^{1/2} < 400$ it seems to transit to the maximum drag reduction asymptote, but at $Re_s f^{1/2} \sim 400$ it breaks away into the polymeric regime, and then for $500 < Re_s f^{1/2} < 2,000$ it describes a straight line, $(S_p, I_p) = (5.0, 3.1)$, roughly parallel to but lying above the Prandtl-Karman law. Qualitatively similar behaviour occurs at 3 and 30 p.p.m. Solutions of 100 p.p.m. show marked shear thinning in laminar flow (power law index 0.60 ± 0.01 for $40 < Re_s < 4,000$) and for $Re_s f^{1/2} > 400$ seem to transit to the maximum drag reduction asymptote, the data points lying nearly parallel to curve (3) for $700 < Re_s f^{1/2} < 1,500$ with horizontal separation corresponding to $\eta_{rel} = 1.5$. So the

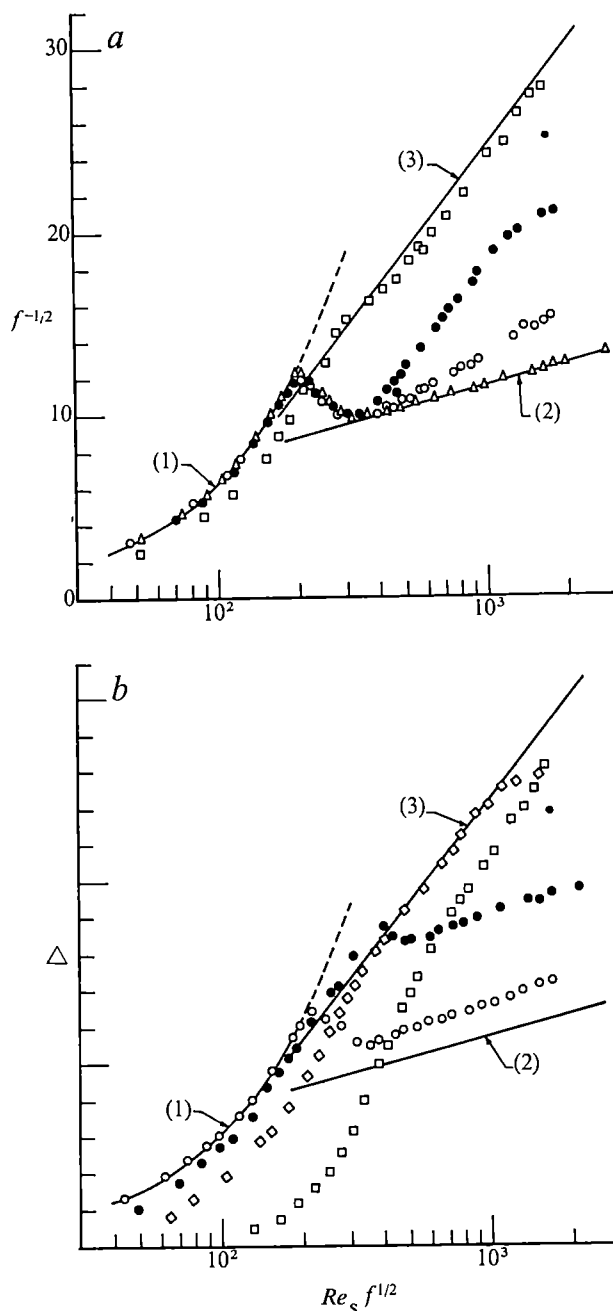


Fig. 1 Drag reduction by solution of a, collapsed polyelectrolyte, b, extended polyelectrolyte. Pipe internal diameter 0.945 cm, length 189 cm, polymer B1110, temperature $21 \pm 2^\circ \text{C}$. The solid lines represent (1) Poiseuille's law (denoted L), (2) the Prandtl-Karman law (N), and (3) the maximum drag reduction asymptote¹ (M). In the region between (2) and (3), called (4) the polymeric regime (P), polymer solutions can be seen to follow approximately linear relationships defined by their slope and intercept (S_p, I_p) . a Δ , Distilled water (DW), \circ 10 p.p.m. in 0.1 M NaCl, \bullet 100 p.p.m. in 1.0 M NaCl, \square 100.0 p.p.m. in 0.1 M NaCl. b \circ 3.0 p.p.m., \bullet 10.0 p.p.m., \star 30.0 p.p.m., \square 100.0 p.p.m. All in DW.

maximum drag reduction possible with extended polyelectrolytes seems to be limited by the usual asymptote, curve (3), with increasing $Re_s f^{1/2}$, the typical solution follows a path sequence LMP which contains a characteristic 'retro-onset' point $Re_s f^{1/2*}$ at which the polymeric regime segment departs from curve (3). Both slope and intercept of the polymeric regime segment increase with increasing polyelectrolyte concentration but all slopes are characteristically close to Newtonian. The LMP trajectory contrasts strikingly with the LNP trajectory typical of random-coiling polymer solutions.

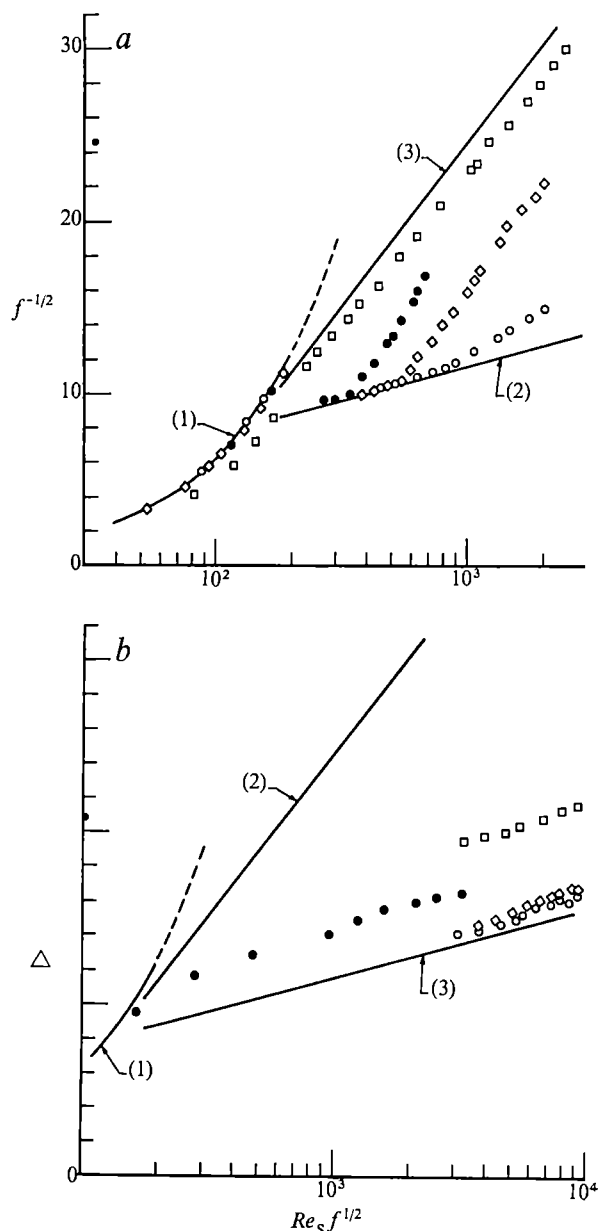


Fig. 2 Drag reduction by *a*, random-coiling polymer solutions, *b*, fibre suspensions⁴⁻⁶ *a* ○, 10.0 p.p.m., W205, ★, 100.0 p.p.m., W205, ●, 10.0 p.p.m., FRA, □, 110.0 p.p.m., FRA. All DW solutions, all pipe 0.945 cm internal diameter except □, 1 d 0.846 cm. *b* ○, 10⁴ p.p.m., nylon, ●, milling yellow crystals, ★, 800.0 p.p.m. asbestos, □, 2,500.0 p.p.m. asbestos ○, 1 d 4.98 cm, ●, 1 d 7.79 cm, ★, and □, 1 d 5.08 cm

Study of 10 p.p.m. B1110 in 1.0, 0.01, 0.001 and 0.0 M NaCl revealed a continuous variation in flow behaviour between the extremes shown in Fig. 1. In terms of drag reduction, measured by fractional flow enhancement $S_F = (-1 + (f_p^{-1/2}/f_s^{-1/2}) Re_s f^{1/2})$, relative efficiencies were $S_F(1.0 \text{ M}) < S_F(0.01 \text{ M}) < S_F(0.001 \text{ M}) > S_F(0.0 \text{ M})$, so a partially extended conformation was 'optimal' for $300 < Re_s f^{1/2} < 2,000$. Relative efficiencies of different conformations were, however, decidedly flow dependent, in Fig. 1 the 1.0 M and 0.0 M NaCl solution trajectories intersect making $S_F(1.0 \text{ M})$ less than, equal to and greater than $S_F(0.0 \text{ M})$ for $Re_s f^{1/2}$ less than, equal to and greater than 1,000 respectively. Possibly, therefore, the apparent conflict between the data of Parker² and of Hand³ may not be serious.

The present results can be related to drag reduction by random-coiling polymer solutions and by fibre suspensions using Fig. 2. Part *a* shows flow data for two polyethyleneoxide (PEO) polymers, FRA, $M = 8 \times 10^6$ and W205, $M = 1.3 \times 10^6$. PEO macromolecules possess a skeletal structure similar to

that of PAMH, and the FRA chosen has roughly the same number of skeletal links, $N \sim 0.5 \times 10^6$, as B1110. There is close correspondence between the 10 and 100 p.p.m. B1110 data in Fig. 1*a* and the 10 and 110 p.p.m. FRA data in Fig. 2*a*. Figure 2*b* illustrates drag reduction by fibre suspensions using data reported for suspensions of a nylon fibre⁴, milling yellow crystals⁵ and two chrysotile asbestos fibres⁶. The fibre suspensions all produce approximately straight lines which lie in the region between curves (2) and (3), possess slopes only slightly greater than the Prandtl-Karman, 4.0, and yield essentially constant drag reduction, independent of $Re_s f^{1/2}$. The milling yellow suspension yields $(S_p, I_p) = (4.5, 0.8)$ and $S_F \sim 0.23$ (constant) for $300 < Re_s f^{1/2} < 3,000$. The gross flow behaviour (Fig. 2*b*) which seems to be characteristic of fibre suspensions, is analogous to that exhibited by extended polyelectrolyte solutions (Fig. 1*b*) in the polymeric regime.

Results obtained with collapsed and extended polyelectrolyte possibly represent two extremes of drag reduction behaviour, one (designated Type A) which is characteristic of flexible, random-coiling, deformable additives and the other, (Type B) which is characteristic of relatively rigid, elongated, undeformable additives. Idealised, a family of Type A solutions yield polymeric regime segments fanning outwards from a common onset point on the Prandtl-Karman line with slopes increasing with increasing additive concentration and drag reduction increasing with increasing $Re_s f^{1/2}$. A family of Type B solutions yield polymeric regime segments coming off the maximum drag reduction asymptote at characteristic retro-onset points and lying roughly parallel to but displaced upwards from the Prandtl-Karman line with drag reduction essentially independent of $Re_s f^{1/2}$ but increasing with increasing additive concentration.

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Novel reactions of hydrocarbon complexes of metal-substituted sheet silicates: thermal dimerisation of *trans*-stilbene

TRANSITION metal, ion-exchanged montmorillonites form complexes^{1,2}, often of an interlamellar kind (intercalates), with a range of aromatic molecules, including benzene, toluene and the xylenes, anisole, and other species such as *trans*-stilbene and indene³⁻⁵. The aromatic molecules can form two main types of complex with the interlayer transition-metal ions, for example Cu(II). In the first type there is thought to be an edge- π -bonded copper arene moiety, similar to the bonding situation which exists in $C_6H_6CuAlCl_4$ (ref. 6), and in the second instance it is considered to be a type of bond which causes associated distortion of the aromatic ring and some localisation of the circumferential double bonds¹. Although detailed crystallographic analyses of the organic intercalates of sheet silicates has only recently commenced (ref. 7, and J. M. Adams *et al.*, unpublished) and in spite of the paucity of information relating to the precise nature of the bonding of organic entities to encaged metal ions, it is clear²⁻⁴ that these organic complexes display a rich range of unusual reactions, some new examples of which we report here.

Complexes of benzene, toluene, the xylenes and *trans*-stilbene were formed with dehydrated Cu(II)-exchanged montmorillonite by exposing the latter to the vapour of each

Table 1 Relative intensities of the mass spectral peaks of the thermal decomposition products.

Toluene complex 150° C		<i>p</i> -xylene complex 150° C		<i>trans</i> -stilbene complex 200° C	
<i>m/e</i>	Intensity	<i>m/e</i>	Intensity	<i>m/e</i>	Intensity
272	17	315	12	450	2
270	18	314	26	449	3
257	18	300	11	372	1
255	20	299	38	370	2
195	10	249	24	360	92
182	13	210	42	346	8
181	19	209	35	344	5
167	16	195	59	280	18
165	23	193	42	279	12
105	25	119	41	270	65
92	62	118	56	269	73
91	106	106	68	252	18
		105	100	239	8
				203	9
				202	13
				191	69
				180	77
				179	76
				178	100

respective aromatic material. Excess *trans*-stilbene was removed by washing with chloroform, and surplus solvent was removed by suction. On heating the complexes *in situ* in an AEI MS30 mass spectrometer, in high vacua, between 50 and 250° C, high molecular weight products appeared.

With the benzene complex of Cu(II) montmorillonite, which is known to involve the second type of linkage between the metal and the aromatic ring, only a negligible proportion of material possessing a molecular weight approximately two or three times that of benzene was produced, whereas with the toluene complex, which has the first type of linkage, appreciable quantities of molecules of mass number 272 and 182 were formed (Table 1). These mass numbers correspond to three toluene units less four hydrogen atoms and to two toluenes less two hydrogens, respectively. Complexes of all three xylenes, which have similar copper-arene linkages to the toluene complex, behaved very similarly and yielded mass numbers of 314 and 210, again signifying the loss of hydrogens when three and two units, respectively, of xylene condense. Blank mass-spectrometric experiments carried out with sodium-ion-exchanged montmorillonite revealed that, essentially, no hydrocarbon of higher molecular weight was formed when traces of physically adsorbed alkyl benzene were heated in the absence of the transition-metal ion.

With *trans*-stilbene, the results were strikingly different in that significant quantities of material possessing a mass number of 360, which corresponds to the dimer, were obtained. (Some ions with *m/e* equal to 370–450 were observed in low abundance, so some trimer or even higher order species must have been formed, although the molecular ion(s) corresponding to such species were not observed.)

Although much remains to be learned about the mechanisms of these thermally induced reactions the obvious major difference in the nature of the products obtained suggests that different reaction pathways are followed depending on whether the organic molecules contain aromatic rings or aromatic rings together with ethylenic double bonds. The implication, which needs to be tested, is that the central olefinic double bond in *trans*-stilbene is bonded to the Cu ion and that, as a result of the proximity and the electronic perturbation of these olefinic double bonds of the two (or more) *trans*-stilbene molecules, dimerisation is facilitated. Though it is unnecessary to postulate that the dimerisation is concerted, it is of interest to note that the thermal production of tetraphenyl-cyclobutane from *trans*-stilbene monomers is, by orbital symmetry, forbidden and that the photochemical dimerisation, though allowed, is not easily achieved either in the solid state^{8,9} or when weakly physically adsorbed¹⁰.

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Systematic position of *Plesiadapis*

THE systematic position of the Palaeocene mammal *Plesiadapis* has been a subject of discussion for almost a century.* This is not surprising considering the meagre evidence on which it was first associated with lemuroid primates. A review of the evidence now available indicates that the affinities of *Plesiadapis* and its relatives are with early tarsier-like primates, and not with archaic lemurs.

Gervais¹ described the genus *Plesiadapis* after he² and Delfortrie³ had recognised *Adapis* as a primate related to living lemurs. Gervais' species *Plesiadapis tricuspidens* was originally based on two specimens collected by Lemoine from the Palaeocene of France—a mandible fragment and an isolated incisor—neither of which show any significant resemblance to *Adapis*. The relationship between *Plesiadapis* and *Adapis* advocated by Gervais was apparently based on several Eocene specimens first described by Lemoine as *Plesiadapis*⁴, but subsequently transferred to the new, genus *Protoadapis*⁵ (which is closely related to *Adapis*).

Once given a name compounded from *Adapis*, no matter how poorly justified that actually was, it is natural that *Plesiadapis* was subsequently compared most closely with lemur-like primates. Simpson⁶ emphasised the detailed resemblance of the molar pattern of *Plesiadapis* to that of the early adapid *Pelycodus*, and classified the Plesiadapidae as a family within the Lemuroidea⁷. The presence of a postprotocingulum ('*Nannopithecus*-fold') on the upper molars of *Plesiadapis*, *Pelycodus*, and early Eocene tarsiid primates is an important derived characteristic shared by early primates⁸ but, considering the important differences now known in the anterior dentition of species of *Plesiadapis* and *Pelycodus*, it appears that Simpson overestimated the significance of their molar resemblances. *Plesiadapis* also differs significantly in middle ear structure from early lemuroid primates.

In a group of mammals with a reasonably good fossil record, a biostratigraphic approach to phylogeny⁹ offers the best evidence for working out the true relationships between species. Virtually all specimens assigned to the family Plesiadapidae have been studied again in a carefully documented biostratigraphic context¹⁰. Five plesiadapid lineages are known, at least two of which were common to both Europe and North America. Particularly interesting here is the major lineage leading from *Prionothodectes matthewi* of the middle Palaeocene (Torrejonian) to *Platychoerops richardsonii* of the early Eocene (Cuisian).

Plesiadapis tricuspidens is a late member of this central lineage. As the earliest plesiadapid and the common ancestor of all the later species known, *Pronothodectes* is the primitive form

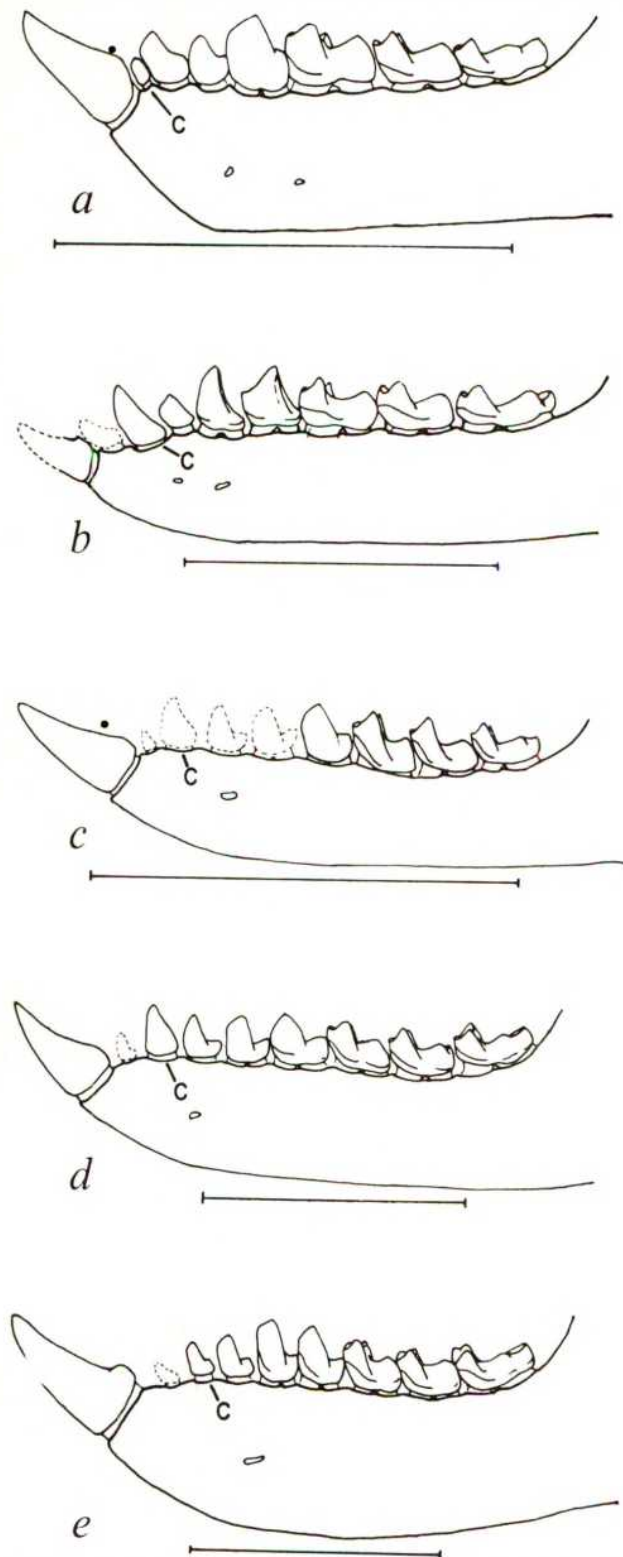


Fig. 1 Comparison of the lower dentition of representative Eocene Tarsiiformes (*Nannopithecus* (a), *Omomys* (b)) with that of middle Paleocene Plesiadapiformes (*Palenochtha* (c), *Plesiolestes* (d), and the primitive plesiadapid *Pronothodectes* (e)). Note enlargement of the central incisor distinguishing these forms from lemuriform and anthropoid primates. c, Lower canine. All figures in lateral view and brought to same size for comparison, bar represents 1 cm. Specimens are a, Halle IL-8, Geiseltalmuseum in Halle; b, AMNH 12600, YPM 13219, 16287, American Museum of Natural History, New York; c, PU 14786, 19461; d, PU 14149, 17427, Princeton University Museum, New Jersey; e, USNM 9332, National Museum of Natural History, Washington, AMNH 35462.

which should be compared with members of other families in determining the relationships of Plesiadapidae.

Pronothodectes matthewi is similar in dental conformation to early species of Carpolestidae, Paromomyidae, and Microsyopidae (Paromomyidae is limited here to forms having tricuspid plesiadapid- or carpolestid-like upper incisors, including *Paromomys*, *Phenacolemur*, and possibly *Saxonella*; whereas the Microsyopidae, including *Plesiolestes*¹¹, *Palaeochthon*, *Palenochtha*, *Berruvius*, *Navajovius*, have simpler bicuspid upper incisors¹⁰.) All have a basic dental formula of

$$\begin{array}{cccc} 2 & 1 & 3 & 3 \\ 2 & 1 & 3 & 3 \end{array}$$

and all have enlarged, procumbent, pointed, lower central incisors. Early Tarsiidae and Omomyidae have the same basic dental formula and very similar lower incisors, with one possible exception: some specimens of *Teilhardina* apparently retained four premolars, although descriptions of the dental morphology of this genus are conflicting and deserve additional study. The lower dental formula of microchoerines is sometimes cited as 1.1.4.3 (ref. 12), but interstitial wear on the medial side of the enlarged anterior tooth shows it to be the central incisor, and the 'alveolus' in front of this tooth is an anterior mental foramen¹⁰.

Figure 1 shows that the mandibular and dental conformation of Palaeocene primates of the infraorder Plesiadapiformes is very much like that of Eocene Tarsiiformes (Plesiadapiformes Simons, 1972¹³ includes the same four families as Paromomyiformes Szalay, 1973¹⁴ and the former name is used here). The morphology of the anterior teeth differs fundamentally from that seen in the earliest lemuroid primate *Pelycodus* and its descendants. The lower central incisor (I_1) of *Pelycodus* is slightly smaller than I_2 and both are considerably smaller than the canine. Furthermore, the incisors of adapids differ in being vertically implanted and in having spatulate rather than pointed crowns.

The auditory bulla and middle ear are exceptionally well preserved in a skull of *Plesiadapis tricuspidens* recently collected by M. Pellouin of Reims from the Palaeocene locality of Berru in France. The auditory bulla in this skull was completely ossified, with no trace of a separate entotympanic element (though an entotympanic centre may have been present during ossification). Russell¹⁵ has described the extended external auditory meatus and fusion of the tympanic annulus into the lateral wall of the auditory bulla in another skull of *Plesiadapis tricuspidens*; the skull illustrated in Fig. 2 shows these characters even more clearly. An extended external auditory meatus and a tympanic annulus fused into the wall of the bulla characterise all Tarsiiformes but not primates of the infraorder Lemuriformes or

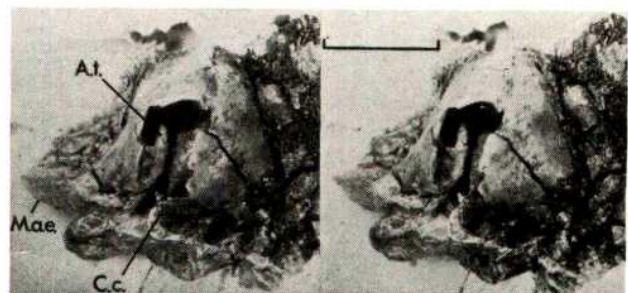


Fig. 2 Right auditory bulla of Pellouin skull of *Plesiadapis tricuspidens* in ventral view (stereophotograph). Note *Necrolemur*-like¹² struts anchoring the tympanic annulus to the lateral wall of the bulla. Fragments were removed from the ventral wall of the bulla to facilitate cleaning. A.t., Annulus tympanicus; C.c., Canalis caroticus; M.a.e., Meatus acusticus externus. This skull of *Plesiadapis* includes an almost complete maxillary dentition, on which its identification is based. Bar represents 1 cm.

primitive Anthropeoidea. The ear region of the skull of *Plesiadapis* (and *Phenacolemur*¹⁶) thus furnishes additional evidence linking plesiadapiform primates to Eocene *Necrolemur*, Oligocene *Rooneyia* and living *Tarsius*, and to the origin of Tarsiiformes.

In both dental conformation and middle ear morphology, generally recognised as the two character complexes of greatest systematic importance among early primates, plesiadapiformes are very similar to early Tarsiiformes and differ greatly from early Lemuriformes. Evidence is presented elsewhere suggesting that Anthropeoidea are derived from lemuriform rather than tarsiiform ancestors¹⁷. Thus there seems, from the fossil evidence, to be a basic dichotomy within the primates separating the infraorders Plesiadapiformes and Tarsiiformes on one hand from the infraorders Lemuriformes and Anthropeoidea on the other^{10,18}. The earliest lemuroid primates appear abruptly in the fossil record, suggesting that they migrated to Europe and North America at the beginning of the Eocene (as did the earliest rodents and several other important groups). *Purgatorius*, from the Early Palaeocene¹⁹, is the only form known which could possibly be the last common ancestor of all later primates.

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Collective orientation in night-flying insects

SEVERAL investigations have been made using microwave radar techniques to study individual insects in free flight^{1–5}. One of the most surprising claims to result from these studies is that insects flying at night sometimes adopt a common orientation, usually downwind^{1,2,4}. This would imply a remarkable ability to determine wind direction when flying in conditions of severely limited visibility¹. We report here an instance of collective insect orientation in nocturnal flight, although in this case, the direction of flight was against the wind.

Our observations were made at Kara in the Niger flood plain in Mali during October, 1973, using radar apparatus

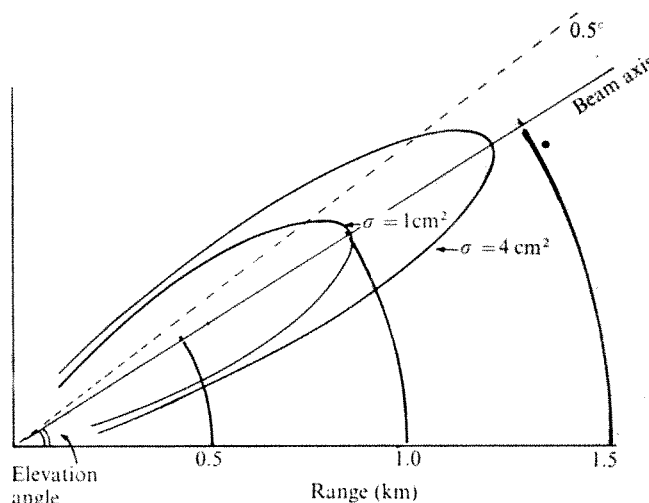


Fig. 1 Typical detection envelopes for two targets of radar cross section (σ) 1 cm² and 4 cm². Targets of these sizes will not be detected outside their respective envelopes. The volume swept out by the envelopes on rotation of the aerial about a vertical axis, and therefore the volumes sampled per revolution for the two sizes of targets, are substantially different. The angular scale in this diagram has been multiplied by 10 to make the effect clear.

modified for entomological observations⁵. The aerial of this radar projects a 'conical' pulsed, microwave beam the axis of which can be set at selected angles of elevation. Rotation of the aerial about a vertical axis causes targets at the appropriate elevations round the radar to be illuminated briefly once per revolution. The resulting radar echoes, if large enough, are registered as 'dots' on a conventional display revolving in synchrony with the aerial. Small targets may be detected at short range and close to the beam axis; larger targets are detectable at greater ranges and further from the axis of the beam. Figure 1 shows typical detection envelopes for two sizes of target. A given airborne volume density of large targets will thus produce more display 'dots' per revolution than the same density of smaller targets.

Measurements made on captive insects indicate that they generally form substantially larger radar targets when the electric vector in the radar wave has a large component parallel to the insect's major body axis^{6,7}. In the case of a radar transmitting horizontally polarised radiation, this means that flying insects will generally present larger 'echoing areas' when flying broadside on to the radar than when end on². A uniform distribution of insects flying with a degree of common orientation in the vicinity of the radar would thus be expected to produce a non-uniform radar display, more 'dots' being seen in the directions from which insects presented predominantly side-on aspects to the radar than in other directions. A pronounced example of this effect is shown in Fig. 2. The distribution of echoes shown in this photograph implies that the insect targets in the vicinity of the radar were predominantly aligned with the 35–215° axis. This 'polarisation' of the echo distribution does not indicate in itself which way along this axis the insects were heading.

Evidence for direction and confirmation of the collective orientation suggested by the polarised display was given by time-lapse photographs of the radar screen. Individual insect echoes were seen to move in the direction of 35° at a ground speed of $\sim 3 \text{ ms}^{-1}$ while the radar echo produced by a freely flying balloon (circled), at the same altitude ($\sim 900 \text{ m}$) and carrying strips of aluminium foil, was displaced towards 215° at $\sim 2 \text{ ms}^{-1}$. The insect heading in this case was clearly against the wind. The aerial density of insects varied from $\sim 50 \text{ per } 10^7 \text{ m}^3$ at 900 m altitude to 20 per 10^7 m^3 at 100 m. The sky was clear with a 7/8 Moon at a bearing of 250°.

The 'polarisation' effect was observed on several nights, being present for 2 or 3% of the observational period (ten nights). The opportunity to provide simultaneous observations

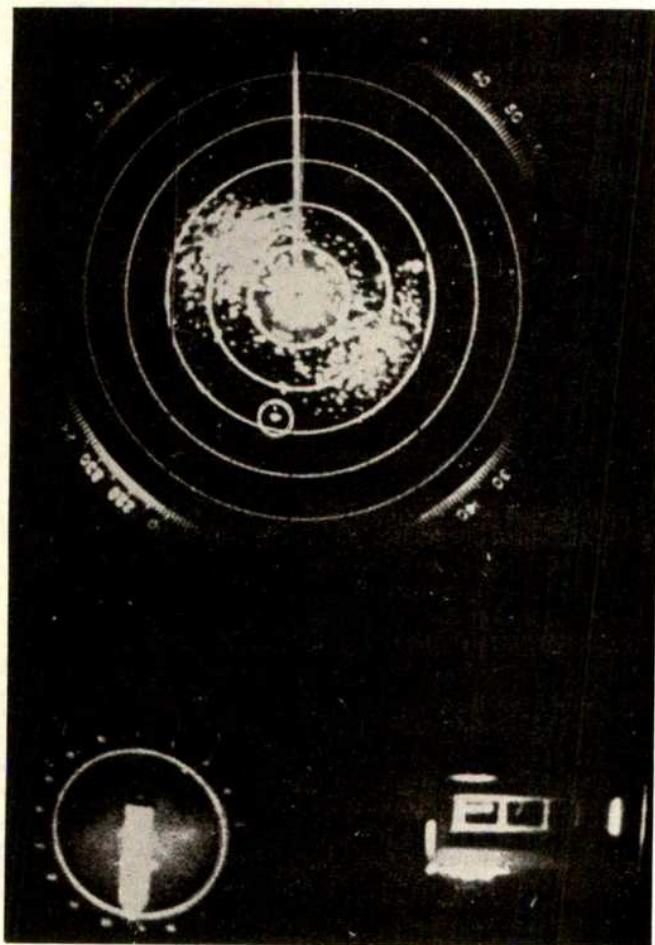


Fig. 2 Photograph of radar screen (1946h, November 3, 1973 at Kara, Mali). Elevation angle 46° , distance between range rings 500 yd. True north is at 0° on the display scale. The echo produced by a freely flying balloon is circled.

of balloon displacement and 'polarisation' did not, unfortunately, recur on these occasions, so that no general implication about heading with respect to wind direction could be inferred. What the observations do show is that some insects regularly demonstrate an ability to adopt a common orientation when flying at night at altitudes of several hundred metres and when separated from each other by distances of the order of 50 m or more.

Information about the identity of insect targets may be gained by measuring the frequency of the modulation produced in their radar echoes by their wing flapping action⁷. Figure 3 shows the distribution of wing-beat frequencies deduced from radar measurements of a sample of the insects responsible for the echoes shown in Fig. 2. Comparison of this histogram with the wing-beat frequencies of other insects found in the

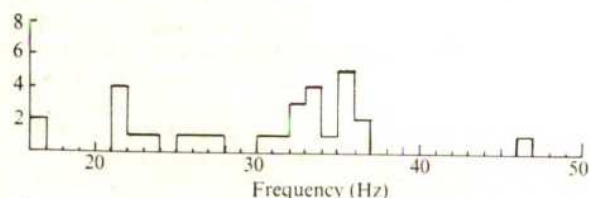


Fig. 3 Histogram showing the distribution of fundamental frequency components detected in radar signatures recorded from targets contributing to the polarised display in Fig. 2. Numbers on the vertical axis indicate the number of signatures containing fundamental frequency components per unit interval of frequency.

area suggests that the echoes may have been caused by grasshoppers, possibly *Trilophidia*, *Oedaleus* and *Locusta*.

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Micromanipulation of stomatal guard cells

THE opening and closing of stomatal pores is controlled by deformations of the guard cells resulting from changes in the pressure relationships between epidermal and guard cells. Under natural conditions such changes are, in turn, controlled by changes in the osmotic concentrations of cell saps or by changes in cell water content, although they could also be controlled by changes in cell wall properties. We have measured the pressures required to bring about or maintain stomatal openings by applying pressures directly within subsidiary cells and guard cells of *Tradescantia virginiana* and *Vicia faba* leaves.

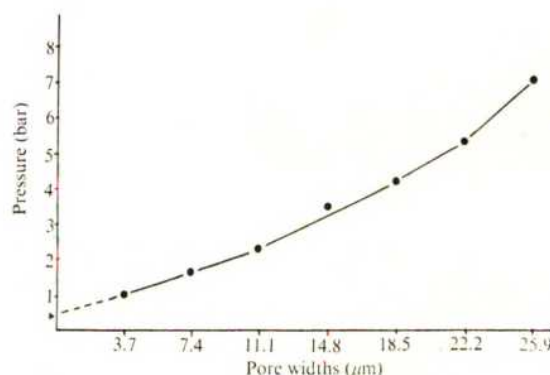


Fig. 1 Pressures applied in subsidiary cells of *Tradescantia virginiana* leaves which caused stomatal closure at various degrees of opening. Each point is the mean of at least 10 measurements with the exception of the values at 3.7 and 25.9 μm where four measurements were made. The pressure values quoted are the total pressures prevailing in the guard cells and not the pressure differentials between epidermal and guard cells. The intercept with the Y axis represents the pressure required to force liquid through the opening of the microneedle. In this species pressures obtained with epidermal strips were of the same magnitude as those with intact leaves. When one of a pair of guard cells was punctured before the application of pressure, the same pressures were required to cause a given degree of opening as with both guard cells intact; this implies that in this species guard cells do not assist each other during movements. Pressures applied in subsidiary cells to close initially open stomata were of the same magnitude as those applied within guard cells to re-open initially almost closed stomata. Until now we have been unable to open completely closed stomata. This observation tends to support Stålfelt's concept of 'Spannungsphase' during which either greater pressures than 10 bar are required before guard cells are deformed; or we must postulate that changes in cell wall properties take place during this phase. The shape of the curve suggests that until an opening of about 10 μm has been reached the deformations of the walls in this species do not involve any substantial stretching of the guard cells. With openings above this size, guard cell walls begin to expand against gradually increasing resistances.

The technique (details will be published elsewhere) consisted of inserting water-filled needles (diameter $<1\ \mu\text{m}$) into the cells and exerting positive or negative pressures using a special syringe attached to the needle. At the same time a transducer was used to measure the magnitude of the pressures, while changes in stomatal pore widths were measured using a microscope with an eyepiece graticule.

Figure 1 shows a summary of the results obtained with *Tradescantia virginiana*. Except during the 'Spannungsphase', operative pressures in the stomatal apparatus were lower than would be expected from some plasmolytically determined osmotic potentials of guard cell saps^{1,2}. Such discrepancies could result from inherent weaknesses in the plasmolytic method. Alternatively, the assumption that guard cells of open stomata (even during steady state openings) are at maximum turgor (in other words, that osmotic potential differences determined plasmolytically cannot be equated to pressure potential differences of guard cells) could be wrong. A mechanism preventing the attainment of full turgor by guard cells can be postulated by reference to the evidence for substantial water vapour loss to the leaf air space from epidermal cells³ and to the outside from guard cells⁴. These possibilities seem to be supported by the comparatively gentle curvature of the line in Fig. 1, suggesting that it is unlikely that these cells were at full turgor. The possibility that guard cell wall properties are unusual and changeable could also be postulated in view of the evidence for ionic migrations between epidermal and guard cells, or between cell walls, the protoplast and vacuoles⁵⁻⁷. These postulates are currently being tested.

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The corneal cones of *Limulus* as optimised light concentrators

In their external shape and optical properties, the crystalline cones of the ommatidia in the compound eye of *Limulus polyphemus* strikingly resemble ideal light collectors. Such devices were developed¹ in a totally different context: for the purpose of optimising the collection of the extremely faint radiation produced in a transparent medium by faster-than-light charged particles (Cherenkov light). Ideal light collectors are ogival-shaped light guides designed to maximise the concentration of diffuse light within a specified angular acceptance. These light guides are optically homogeneous and derive their characteristic properties from the specific shape of the exterior surface which is made specularly reflecting.

Figure 1 illustrates the performance of an ideal light collector. All light rays incident upon the entrance pupil S_1 at an angle $\theta < \theta_{\max}$ are channelled through the exit pupil S_2 after undergoing perhaps one or more reflections. The rays emerge with a large angular spread, approaching 90° to the cone axis. For the

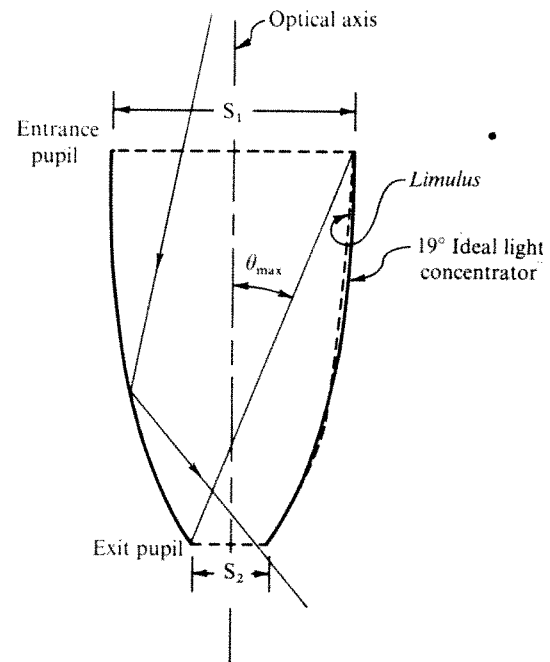


Fig. 1 Profile of ideal light concentrator for maximum angle of acceptance $\theta_{\max} = 19^\circ$. All light rays incident upon the entrance pupil within a cone of aperture θ_{\max} are collected and conveyed to the exit pupil. On the right branch of the profile, the shape of a crystalline cone of *Limulus* is also shown.

ideal collector, the concentration factor $x = S_1/S_2$ is related to the angular acceptance θ_{\max} by

$$x = 1/\sin^2 \theta_{\max} \quad (1)$$

This performance is characteristic of an optical system with $f/\text{number} = 1/2$, the theoretically lower limit to the relative aperture imposed by geometrical optics. A complementary property of such systems is to exclude any stray light incident at angles greater than θ_{\max} .

The qualitative similarity between the profile shape of an ideal light concentrator (Fig. 1) and that of the crystalline cones of *Limulus* became apparent from a micrograph^{2,3} of the anatomy of the compound eye of this Xiphosurid. A quantitative match of the profile shape of several crystalline cones with theoretical shapes corresponding to various acceptance angles θ_{\max} showed a very good fit for $\theta_{\max} = 19^\circ$. This is in fact the value of the parameter θ_{\max} for the profile shown in Fig. 1, where the comparison with the shape of a typical crystalline cone of *Limulus* is indicated. Cone shapes corresponding to smaller acceptance angles occur near the periphery of the eye.

The simplest optical mechanism by which the *Limulus* cones could function as ideal light concentrators involves total internal reflection of the incident light at the interface between the crystalline cones and the surrounding media. For an optically uniform cone of refractive index $n = 1.54$ (refs 4 and 5) in optical contact with tissue fluid of $n = 1.34$ (ref. 5), the collection of light would be highly efficient, since only a small fraction of light rays would impinge on the internal cone wall at angles in excess of the critical angle (nearly 60°) and leak out. A similar analogy between the ellipsoid portion of retinal cone cells and ideal light collectors has already been noted⁶.

Since the crystalline cones, protruding inward as part of the corneal cuticle, are embedded for about two-thirds of their length in a transparent matrix whose optical properties have never been critically analysed, it was necessary to test the assumptions of our model. The lateral eyes of adult female specimens of *Limulus* were excised and the cornea cleaned of the rhabdomes and pigmented tissue. Throughout all observations, the specimen samples were kept immersed in the original tissue fluid. A portion of the interior visual surface thus prepared is shown in Fig. 2a, where the resemblance of the cone

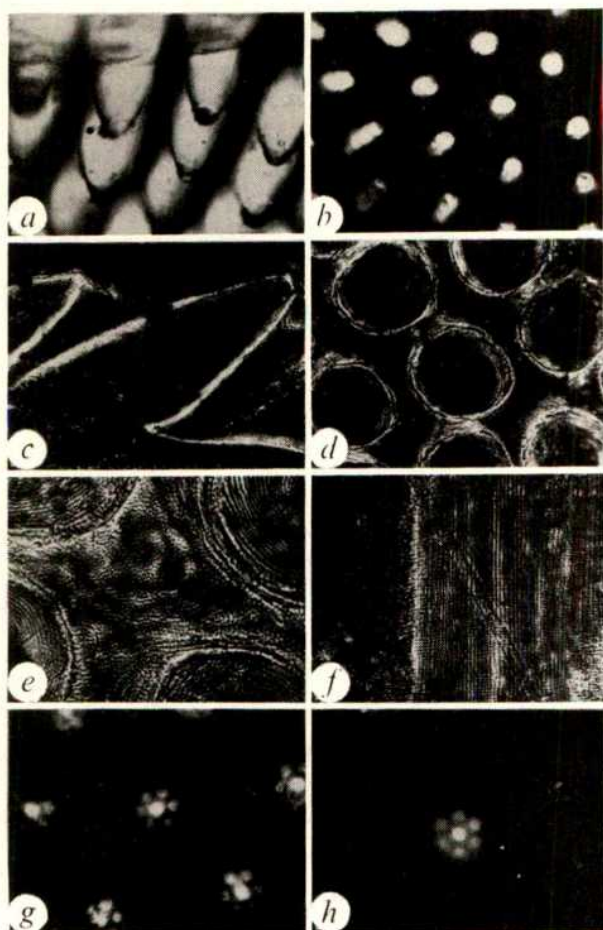


Fig. 2 *a*, Interior surface of the central area of the cornea in the lateral (compound) eye of *Limulus polyphemus*. Fresh specimen, immersed in tissue fluid ($\times 33$). *b*, Interior of the visual surface, as in *a*, when illuminated by a diffuse source from the exterior side of the cornea. The light entering the crystalline cones is channelled to their tips ($\times 33$). *c*, Interference contrast micrograph of the *Limulus* cornea. Fresh section ($10\ \mu\text{m}$ thick) normal to the exterior surface near the periphery of the eye. The section is immersed in tissue fluid. A bright fringe outlines a crystalline cone, whose refractive index is higher than the intracrystalline fill ($\times 55$). *d*, Interference contrast micrograph, as in *c*, of a section parallel to the corneal surface. Bright circular fringes define the cross sections of the crystalline cones ($\times 55$). *e*, Enlarged view of fresh section of the corneal matrix filling the space between crystalline cones. The cross section of the pore canals, filled by tissue fluid is made visible by interference contrast ($\times 133$). *f*, As in *e*, in a longitudinal view. The darker area on the left is part of a crystalline cone ($\times 133$). *g*, Images of a grid (mesh opening $3\ \text{mm}$) placed in front of the visual surface at a distance of $2.6\ \text{cm}$. The images are focused at the tips of the crystalline cones, when the latter are immersed in water. The field of view subtended by each cone belonging to the central region of the eye, is approximately 20° ($\times 48$). *h*, Image obtained at the tip of a lucite macroscopic replica of a *Limulus* cone, having the theoretical profile shape of ideal light concentrators. Lucite cone immersed in water ($\times 38$).

shapes with the profile of Fig. 1 can clearly be seen. The gross light-concentrating properties of the crystalline cones are illustrated in Fig. 2*b*. Even in the absence of pigment, they behave as light guides, concentrating the light incident on the external surface of the cornea on to the tips of the cones, where the rhabdomes are normally attached. Frozen sections ($\sim 10\ \mu\text{m}$ thick) were then observed with a Baker double focus interference microscope. The crystalline cones preserved their optical identity throughout the entire depth of the cuticle. Their shape is clearly outlined (Fig. 2*c* and *d*) by a bright fringe marking a decrease in refraction index from the interior of the cones to the embedding matrix. All corneal material consisted of cuticle laminations separated by layers of fluid, local variations in the average refractive index, such as that at the cone boundary, arising from variations in the spacing and thickness

of such laminations. The cone interior has a remarkably uniform refractive index, as previously shown⁴. Although the gross refractive properties of the intracrystalline fill can be described in terms of an average measured refractive index $n \sim 1.48$, closer inspection reveals a highly inhomogeneous structure for this tissue (Fig. 2*e* and *f*). It has a loose fabric, permeated by pore canals⁵ running normally to the cuticle laminations. Tissue fluid in the matrix of pore canals seems effectively to constitute a liquid sheath surrounding the highly refracting crystalline cones. The relevant refractive indices for total internal reflection are then those of the cone and tissue fluid respectively, as previously considered⁵.

The phenomenon known as 'pseudopupilla'⁷ is readily explained in our model. All light entering the ommatidium through a cone with angular acceptance θ_{max} is collected and does not re-emerge. In these conditions, the entrance pupil seems a velvety black: the ommatidium behaves as a light sink. Moreover, our simple model of a homogeneous, highly refracting crystalline cone is capable of accounting for the remarkable imaging ability of the crystalline cones observed by many investigators⁷. It is precisely this imaging property (Fig. 2*g*) which prompted earlier attempts to invoke complicated mechanisms for their optical function. A model of the crystalline cone consisting of a lucite light guide ($n=1.5$) with the ideal collector profile immersed in water ($n=1.33$) reproduces the images observed at the tip of an actual cuticular cone of *Limulus*.

Several assumptions have been made in our analysis. The laminated structure of the crystalline cones and the finite thickness of the optical discontinuity at the cone boundaries will make the light collector somewhat inefficient, particularly towards the cone tip. The extent of this light leakage can be appreciated from Fig. 2*b*. In *Limulus* the problem is overcome by encapsulating the distal part of the cone in a sleeve of cells bearing reflecting-pigment, the guanophores⁸.

We conclude that the ommatidia of *Limulus* provide a good example of an optimisation process in nature. Among the possible advantages to an organism that evolves ideal light collectors, the most important is probably the ability to see in dim light conditions.

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Recovery of haemolytic plaque-forming cells after freeze drying

FREEZE drying is generally accepted as the most convenient and often the best method for the preservation of bacteria and viruses. With mammalian cells, the situation is different and

cells such as lymphocytes have to be stored in liquid nitrogen, a method that is cumbersome and inconvenient and which makes transport of such cells particularly difficult. Up to the present, there has to our knowledge been only a single report of successful recovery of function following rehydration of a freeze dried nucleated mammalian cell. This was by Meryman and Kafig in 1959¹ using spermatozoa, but neither they² nor other workers³ have been able to repeat this work. Freeze drying of non-nucleated mammalian cells, such as erythrocytes, has been more successful, however, and both Meryman⁴ and Greaves⁵ have reported the lyophilisation of small numbers of these cells.

The use of cryoprotectants such as dimethylsulphoxide (DMSO) or glycerol are commonplace in the cryopreservation of cells. These substances penetrate the cells and obviate freezing damage probably because of their ability to 'buffer' salt concentration by their colligative properties⁶. When freeze drying is carried out, these substances being non-volatile become concentrated at levels toxic to most cells²⁻⁶. The use of polyvinylpyrrolidone (PVP) in conjunction with foetal calf serum (FCS) has been investigated⁷. PVP does not penetrate the cell and following the previous work it seemed worthwhile to try to use it to protect cells against damage by freeze drying. Here we describe the successful recovery of a lymphocyte function following freeze drying of cells from a medium containing PVP and FCS. The function we chose to investigate is the formation of haemolytic antibody (IgM) as demonstrated by the production of plaques of haemolysis in thin layers of sheep red blood cells (SRBC) by spleen cells from mice previously sensitised with SRBC. This was chosen because of the high sensitivity and specificity of the test.

For each experiment, six female mice (Porton albino) were each injected intravenously with 5×10^8 SRBC. Four days later, the spleens were removed and homogenised by gentle sieving through a nylon sieve. The cells were washed with Medium 199 by centrifugation for 10 min at 190g and were resuspended in Medium 199. The total number of white cells was counted in the normal way in a haemocytometer and the cell density of the suspension adjusted to 5×10^6 ml⁻¹. The number of plaque forming cells (PFC) was estimated by the method of Cunningham and Szenberg⁸. Aliquots of the cell suspensions (5 ml) were distributed in centrifuge tubes, spun for 10 min at 190g and resuspended in one of two previously prepared cryoprotective media. The first was 10% (v/v) DMSO in Hanks solution, the second was 14% (w/v) dialysed PVP (average molecular weight 24,500; Koch-Light Laboratories Ltd., Colnbrook, UK) supplemented with 30% (v/v) FCS in Hanks solution⁷. In preliminary experiments with mouse spleen cells this medium was examined at pH 7.2 and 6.0 and the latter was found to be better for freeze drying. This may have been because the rise in the pH value due to loss of CO₂ from the serum⁹ was balanced by the lower initial pH. In the results presented the pH of the PVP/FCS medium was adjusted to 6.0 as a routine.

For freezing and drying, 10-ml vials (neutral necked vials, Specification no. 1 (M) 3479, Johnsen and Jorgensen Ltd, London) were used, freezing being carried out in 1-ml amounts and freeze drying in 0.1-ml amounts. The samples were cooled slowly (approximately 2° C min⁻¹) on the pre-cooled shelves (-40° C) of the EF6 freeze drier (Edwards High Vacuum Ltd)

Table 1 Recovery of total number of mouse spleen cells and plaque-forming cells after freezing and freeze drying in 10% DMSO

Cell suspensions	Total white cell recovery		PFCs	
	No. cells (ml ⁻¹ × 10 ⁶)	%	Total no.*	%
Control	5.0	100	1,380	100
Frozen and thawed	3.9	78	147	10.6
Freeze dried and rehydrated	0.75	15	0	0

* Each value is average of counts in two chambers.

Table 2 Recovery of total number of mouse spleen cells and PFCs after preparative manipulation and after freezing and freeze drying in the medium containing 14% PVP and 30% FCS

Cell suspensions	Total white cell recovery		PFCs	
	No. cells (ml ⁻¹ × 10 ⁶)	%	Total no.*	%
Control	5	100	1,090	100
Untreated cells after preparative manipulation	2.3	46	510	46
	1.9	38	465	42
	1.7	34	495	45
Frozen and thawed	1.9	38	155	14
	2.2	44	145	13
	1.5	30	130	11
Freeze dried and rehydrated	2.1	42	130	11
	1.5	30	150	13
	2.3	46	170	15

Control suspension contained cells in Medium 199, the cell density being adjusted to 5×10^6 cells ml⁻¹, 5 ml being examined. The cell suspensions in the PVP/FCS medium were divided into three groups and treated as follows. First, cells from three 5 ml samples were centrifuged again and resuspended in Medium 199. This was devised as a control for the manipulation other than freezing and drying. Second, cell suspensions from three 5 ml samples were distributed in vials and frozen in 1 ml mounts; this was the control for freezing without drying. Third, suspensions from three 5 ml samples were dried in 0.1 ml amounts. After rehydration, pooling in 5 ml amounts and subsequent centrifugation, the pellets of these samples were resuspended in Medium 199. All the experimental groups were examined in triplicate.

* Each value is average of counts in two chambers.

down to -38° C and -40° C and then removed and thawed quickly in a water bath at 37° C (1-ml samples) or freeze dried (0.1-ml samples). For the latter, as soon as a vacuum of 0.07-0.09 torr had been obtained, the temperature of the shelves was raised slowly (2 h) to -20° C, and drying carried out at this temperature for 2 h. The vials were then cooled again to -40° C, dried for an additional 20 h and sealed under vacuum with rubber stoppers. This technique resulted in a residual moisture of 8-10% which was determined by a microdrying method¹⁰. To rehydrate the cells, 0.1 ml of cold Medium 199 was added on the surface of the freeze dried cake, the vials being kept at -40° C. After the medium had frozen the vials were removed from the cold shelves and stood at room temperature to allow the ice to melt. Thus the cake was rehydrated at the temperature of melting ice. The thawed and rehydrated samples were pooled in 5.0-ml amounts, centrifuged and resuspended in Medium 199. The total white cell counts and the number of PFCs were then determined.

Table 1 shows the results of tests when DMSO was used as a cryoprotectant. 78% of the total number of cells was recovered from the freezing and thawing process, while only 10% of the original PFC survived. When the cells were rehydrated following freeze drying, only 15% of the total cells were found and not a single PFC. These results are typical of our experience with the use of DMSO. It is a satisfactory cryoprotectant as far as freezing is concerned but it is toxic when used in a process involving freeze drying.

Table 2 shows the results obtained in an experiment in which PVP and FCS were used to protect the cells. In several experiments, we had found a substantial loss in the total cells and PFCs during the manipulations before either freezing or freeze drying. Such a loss is shown in Table 2. In spite of this, however, it is clear that 30-40% of the total cells have been recovered from the complete process, while 11-15% of the PFC have come through. If the loss of cells due to the manipulations is taken into account, the recovery of total cells from the freeze drying process becomes nearly 100% and the recovery of PFC nearly 30%. No change of total cell numbers or PFC was noticed after 7 d storage at 4° C.

Having thus established that cells dried in the presence of DMSO would not produce haemolytic plaques whereas those

freeze dried from PVP would, we decided to investigate various properties of the plaques. In particular, we wished to know whether the plaques were produced by antibody; whether antibody was secreted or diffusing; or whether antibody synthesis was involved.

The results shown in Table 3 pertain to the first two questions. The total abolition of plaques by azide, found on more than one occasion, shows that plaque formation is energy dependent and thus not the result of simple leakage of preformed material from damaged cells. That the plaques are formed by IgM antibody is shown by the facts that no plaques are formed in the absence of complement, that plaque formation is inhibited by dithiothreitol and that anti- μ -chain serum which is immunologically specific for the μ chain of mouse IgM molecules also blocks plaque formation. Thus, the results show that plaques are formed by IgM molecules secreted from cells by a process requiring energy and, as haemolysis occurs when sheep red blood cells are the target cell and not when chicken erythrocytes are used as a target, it is clear that the required specificity of the antibody exists and demonstrates that the plaques are due to specific IgM and are not an artefact.

Table 3 Haemolytic plaque formation by rehydrated freeze dried cells

Experiment	Total white cell count (ml ⁻¹ × 10 ⁶)		PFCs*					
	1	2	1	2	2a	2b	2c	2d
Control	5.0	4.8		1,100				
		4.7	435	965				
		5.1	1,035					
Untreated cells after preparative manipulation	2.3	1.8	205	425				
	2.8	1.2	210	485				
	3.0	1.4	230	375				
Freeze dried and rehydrated tested as Exp. 1	1.4							
	1.1	—	0	—	—	—	—	—
	1.4							
Freeze dried and rehydrated	1.8	1.1	345	135				
	2.0	1.4	325	130	0	0	0	0
	1.7	1.0	380	100				

Experimental details are as given in Table 2. In experiment 1, 10⁻¹ M azide was added to one group of chambers. In experiment 2, the following variations were used: 2a, the chambers contained no complement; 2b, the chambers contained chicken red blood cells in place of sheep red blood cells normally employed; 2c, the chambers contained 1 drop of 1:2 dilution of rabbit anti-mouse μ -chain serum; 2d, the chambers contained 10⁻¹ M dithiothreitol.

* Each value is average of counts made in two chambers.

Further information on the level of cellular integrity was sought by the use of an inhibitor of protein synthesis, in this case puromycin. This antibiotic causes chain termination by forming a peptide bond with the nascent polypeptide forming on the ribosome¹¹. In a preliminary experiment, puromycin at 10⁻⁴ M inhibited PFCs by about 60%. A further experiment was set up, the results of which are shown in Table 4, from this it can be seen that puromycin at 10⁻² M failed to give complete inhibition of PFC in either the control or the prefrozen and manipulated samples, giving in fact a reduction to around 17% and 10% respectively (with 10⁻³ M puromycin, the figures were 70% and 60%). In the case of freeze dried and reconstituted cells, there was virtually no inhibition at 10⁻³ M but the 10⁻² M concentration gave a reduction to about 40%. These results would seem to indicate that there has been a selection of non-puromycin sensitive cells following freeze drying but that there is still a proportion of these cells in which some degree of protein synthesis, that is, production of nascent polypeptide chains, is occurring.

We have also considered the freeze drying aspects of this problem. The moisture levels reported here are high relative to the standards accepted in microbiology. It should be noted, however, that the 'microdrying' method for moisture determination used in the present study is based on the loss in weight of a

Table 4 Effect of puromycin on plaque formation by mouse spleen cells

Experiment	Total white cell count (ml ⁻¹ × 10 ⁶)	PFCs*		
		Normal	10 ⁻³ M puromycin	10 ⁻² M puromycin
Control	5.6	5,740	3,665	1,150
	6.3	6,135	4,335	1,020
	6.7	5,485	4,165	870
Untreated cells after preparative manipulation	4.8	2,835	1,725	285
	5.0	3,540	2,485	475
	4.4	3,335	2,590	205
Freeze dried and rehydrated	3.4	1,155	1,180	535
	4.7	1,685	1,295	565
	3.9	1,245	1,190	530

* Each value is average of two counts made in two chambers.

5–10 mg sample on drying in a vacuum at an elevated temperature (80–100° C) and is said to give considerably higher moisture contents than the so-called American standard method¹⁰. Further, we have demonstrated experimentally that even at moisture levels of 2.7% as determined by the Baker method¹⁰, PFC can still be found although at lower recovery rates, 25% of PFCs in control compared with 45% at 11% moisture. The inability of hitherto reported mammalian cells to withstand dehydration to a water content below about 25% (refs 12 and 13) renders our results even more encouraging.

We have also been able to show that freeze dried cells with a residual moisture content of around 5% can be stored for up to 3 weeks at room temperature and still retain their capacity to produce plaques.

The number or proportion of PFCs recovered following freeze drying is currently very variable and at present we have no satisfactory explanation of this fact. Analysis of results from six separate experiments and two storage experiments shows that the results were variable. Geometric means and standard deviations were calculated from log transformed data. The geometric means found (shown with their s. d.) varied from as few as 6.1^{+4.9} PFCs recovered from 197.9^{+27.6} PFCs to as many as 35.2^{+4.9} PFCs from 37.2^{+7.9} PFCs. Most results, however, are not as extreme as these and suggest that a mean recovery of 10–15% of the PFCs in the original spleen cell suspension is found after all manipulative and freeze drying losses. The loss due solely to freeze drying and rehydration is difficult to assess now. The puromycin experiments indicate that some selection of cell population occurs during these processes.

Our experiments suggest the inadequacy of the more commonly used intracellular cryoprotectant agents, such as glycerol and DMSO, for protecting cells against the effects of freeze drying. These agents concentrate within the cell during the sublimation of the water and reach levels that are toxic to the cells^{3,6}. With DMSO in our study, not a single PFC survived. It would seem, therefore, that only those mammalian cells which can survive freezing without glycerol or DMSO may be potential candidates for freeze drying. The extracellular protective medium used here has recently been developed by us⁷ and it has been shown to protect effectively human lymphocytes against freezing damage.

The haemolytic effects seen in our experiments are shown to be due to the active secretion of complement-fixing, antigen-specific IgM molecules. At least some cells seem to be involved in polypeptide synthesis on the ribosomes. Thus, a degree of functional integrity of the cell has been demonstrated and we believe therefore that the work we have described represents a novel and important step towards the recovery of fully functioning nucleated mammalian cells following freeze drying. It may be noted also that for the first time drying-rehydration injury has not been of a higher degree than freezing-thawing injury. So far even in the preservation of micro organisms, freeze drying has always been more deleterious than freezing¹⁴.

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Evidence against interconversion of microtubules and filaments

MICROTUBULES, hollow cylindrical structures of diameter ~ 250 Å and variable length, consisting of 13 protofilaments (diameter ± 40 Å), are now generally accepted as an essential constituent of most eukaryotic cells. They have been implicated in such diverse functions as: maintenance of cell shape, intracellular transport and organisation, movement (particularly slow morphogenetic movement) and control of cell surface topography (for review see ref. 1).

In general, cytoplasmic microtubules are intermingled with filaments (diameter ~ 100 Å), and it has been suggested that they are two components of one functional system. The (supposedly) rigid microtubules would then serve as rails along which organelles are transported through interconnecting filaments². Numerous observations both *in vivo* and in cell culture systems have shown that disruption of microtubules is followed by the appearance of large amounts of filaments often arranged in tightly packed bundles^{3–7}. This has usually been interpreted as an interconversion of microtubules into filaments (refs 3 and 8). Other authors, however, favour the idea that depolymerisation of microtubules would result in a redistribution of existing filaments from a dispersed status to an aggregated form⁹.

Unfortunately, the very nature of these structures does not make it feasible to perform accurate morphometric studies which could solve the problem. We have compared the effect of different antitubulins at different dose levels and with prolonged incubation times, in particular the vinca alkaloids, considered to be very useful in this context, since these drugs are able to precipitate microtubular protein as crystalline aggregates at higher dose levels. We have tested the following hypotheses: (1) If microtubules and filaments are composed of the same precursor protein then there should be an inverse relationship between filament whorls and 'microtubular' crystals in cells treated with vinca alkaloids. (2) If there is a redistribution (aggregation) of filaments after microtubular disruption then these filaments should be absent in the zones between the bundles. (3) If the occurrence of bundles is due

to enhanced synthesis of filaments then this should be largely inhibited by inhibition of protein synthesis.

Cells from a C3H mouse embryonal cell line (MO; ref. 10) show contact inhibition of both movement and division, and form transparent sheets of large polygonal cells at confluency. When these cells were treated with antitubulins* (colchicine, vinblastine and vincristine) they lost their characteristic stretched appearance and assumed a flattened round form. This coincided in time with the disappearance of microtubules at the ultrastructural level, and with a total loss of intracellular orientation and compartmentalisation. This was most obvious in the organisation of the Golgi field which has a perinuclear position around the centrioles in untreated cells and 'explodes' completely after microtubule dissolution, with individual Golgi organelles being distributed over the entire cytoplasm; and the centrioles often assume a peripheral position. Both subplasmalemmal microfilaments and 100 Å filaments were obviously unaffected. The foregoing took place within 10 to 80 min, depending on the dosage used ($100\text{--}0.04\text{ }\mu\text{g ml}^{-1}$). After 2 h a proliferation of smooth endoplasmic vesicles occurred, which later subsided, followed by a general hypertrophy of the rough endoplasmic reticulum and the appearance of annulated lamellae. Increased amounts of 100 Å filaments were observed after only 5 h, bundles and whorls of which continuously increased in size and number, and reached a maximum after 24–48 h (Fig. 1a). The zones between the bundles still contained individual filaments.

When cells were treated for prolonged periods (24–48 h) and subsequently replaced in normal growth medium they regained their normal stretched appearance within 24 h. At the ultrastructural level this coincided with the reappearance of microtubules, beginning after about 4 h, and consequent intracellular reorganisation. Within 24 h the centrioles reassumed their normal perinuclear location in the centre of the regrouped Golgi zone. Filament whorls diminished in size and number, however, at a very slow rate, large aggregates still being observed after 48 h. Both the rate of appearance of filament accumulation after treatment and the disappearance after discontinuation of treatment can hardly be explained by either interconversion of microtubules in filaments or a mere redistribution of pre-existing filaments.

We tried to check the most likely explanation: an enhanced synthesis of filaments after microtubule dissolution. Cells were pretreated with cycloheximide ($10\text{ }\mu\text{g ml}^{-1}$) followed by colchicine ($1\text{ }\mu\text{g ml}^{-1}$) after 16 h. In this situation (which produced a 95% inhibition of labelled leucine incorporation into acid precipitable material; M.D.B., unpublished), the cells reacted in the same way, except for the total lack of filament whorls even after 48 h of treatment (Fig. 1b). Individual filaments could, however, be observed throughout the cytoplasm. Additional evidence against interconversion of microtubules and filaments was obtained by treating the cells with vinca alkaloids. Both vinblastine and vincristine produced effects identical to those of colchicine, filament bundles occurring in the same amount at all doses tested ($10\text{--}0.1\text{ }\mu\text{g ml}^{-1}$). At doses above $1\text{ }\mu\text{g ml}^{-1}$, however, an additional feature appeared: the occurrence of large crystalline aggregates, with a tubular substructure, most prominent at $10\text{ }\mu\text{g ml}^{-1}$. These structures, known to be composed of tubulin^{8,11}, did not interfere in any way with the concomitant presence of filament whorls (Fig. 1c).

These results show that neither interconversion of microtubules in filaments nor redistribution of pre-existing filaments are sufficient explanations for the appearance of large filament aggregates in MO cells after microtubule dissolution. Moreover, it can hardly be attributed to a side-effect of the drug used, since it is produced by all known antitubulins regardless of their often totally different chemical nature. The most likely explanation is obviously an excessive synthesis of filamentous protein.

Although the function of these filaments has not yet been completely elucidated, they always occur in close connection

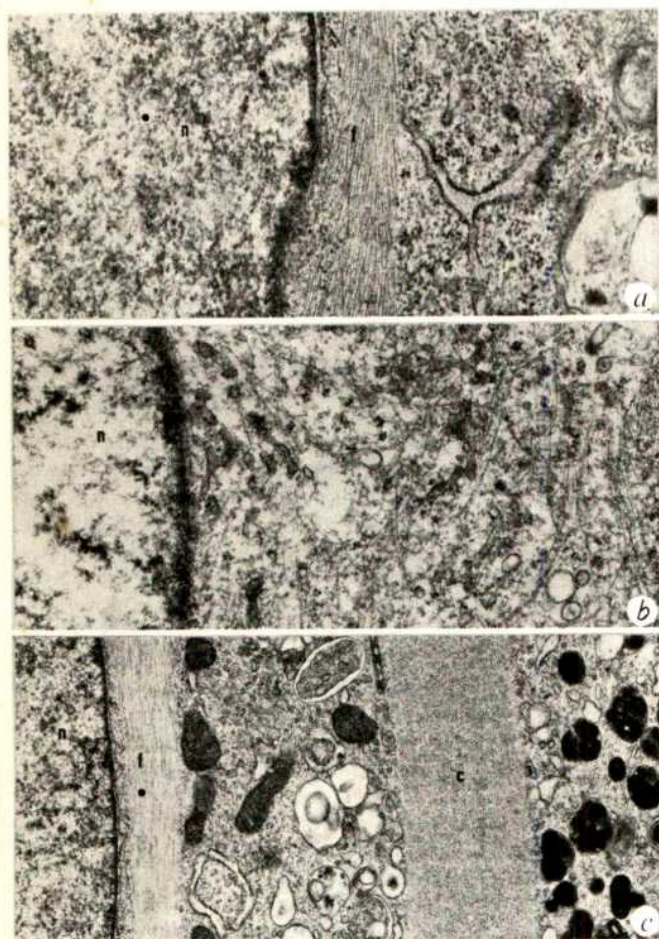


Fig. 1 *a*, Bundle of filaments (*f*) in the vicinity of the nucleus (*n*) in a MO cell treated for 24 h with colchicine ($1 \mu\text{g ml}^{-1}$) ($\times 26,000$). *b*, Perinuclear region in a MO cell treated for 24 h with cycloheximide ($10 \mu\text{g ml}^{-1}$) followed by colchicine ($1 \mu\text{g ml}^{-1}$) after 16 h. Filament bundles are completely absent although individual filaments are present. ($\times 22,000$). *c*, Perinuclear region in a MO cell treated for 24 h with vinblastine ($10 \mu\text{g ml}^{-1}$). Both filament bundles (*f*) and crystalline structures (*c*) are present. ($\times 11,000$). The cells were cultured in Eagle's minimum essential medium supplemented with 10% foetal bovine serum. For observation with the phase contrast microscope and subsequent fixation for electron microscopy the cells were cultured in Falcon plastic Petri dishes (6 cm ϕ). At the appropriate moment the medium was decanted and the cultures washed twice with saline at room temperature. The cells were fixed with 5% glutaraldehyde in 0.1 M sodium cacodylate at room temperature for 15 min and postfixed with a mixture of 4% glutaraldehyde and 1% osmium tetroxide at 0°C . After dehydration in a graded alcohol series the cells were embedded in epon. Under the phase contrast microscope individual cells were selected for further processing for electron microscopy. A minimum of 50 cells was examined per treatment group, each cell being sectioned in a semi-serial fashion from bottom to top, to exclude major sampling errors.

with microtubules in untreated cells. On the other hand, they frequently seem to make contact with organelles such as mitochondria and lysosomes^{12,13}. This has led to the supposition that filaments, together with microtubules, could be responsible for the intracellular directional movement of these organelles^{2,14,15}. A possible hypothesis is that the increased production of filaments after microtubule dissolution could be due to some feedback mechanism whereby the loss of one component of the system (microtubules) is, to some extent, overcome by the hypertrophy of the other component, which could presumably take over some of the lost functions.

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Morphine antagonises action of prostaglandin in neuroblastoma and neuroblastoma \times glioma hybrid cells

WORK on cell lines derived from tumours of the nervous system has been carried out to establish models for neurones and glial cells. Clonal cell lines derived from mouse neuroblastoma C 1300 possess many properties characteristic of neurones¹⁻¹¹. While the neuroblastoma cells increase in their intracellular levels of adenosine 3',5'-cyclic monophosphate (cyclic AMP) in the presence of prostaglandin E_1 (PGE_1) (refs 12 and 13), acetylcholine¹⁴ and adenosine¹⁵, rat glioma cells¹⁶ do so when exposed to noradrenaline^{17,18} or PGE_1 (ref. 19).

Hybrids between mouse neuroblastoma and rat glioma cells display remarkable neuronal properties. In contrast to their parental lines, they contain choline acetyltransferase^{20,21}. They are rather large cells capable of extending long processes; they fire action potentials in response to depolarisation by electrical current or acetylcholine²¹, they contain clear and dense core vesicles²² and have dopamine- β -hydroxylase activity⁵; they increase their content of cyclic AMP in the presence of PGE_1 (ref. 19).

In the course of our studies on the action of PGE_1 on neuroblastoma cells and their hybrids, we investigated²³ the potency of substances, which had been reported^{24,25} to interfere with the influence of prostaglandins. Among these we examined morphine²⁶, as the opiate is known to inhibit the contractions of intestinal muscle^{25,27,28} evoked by PGE . Here we report that morphine antagonises the stimulatory action of PGE_1 on the level of cyclic AMP in neuroblastoma and neuroblastoma \times glioma hybrid cells. While this work was in progress²⁶, Collier and Roy reported that morphine inhibited the stimulation by PGE_1 of the formation of cyclic AMP in rat brain homogenates²⁹.

The neuroblastoma¹³ and neuroblastoma \times glioma hybrid¹⁹ lines which are most responsive to PGE_1 were cultured in plastic dishes 10 cm in diameter. For experimental incubation, the cells were kept for 10 min at 37°C , at varying concentrations of morphine in the absence or presence of $10 \mu\text{mol l}^{-1}$ PGE_1 as described previously²³. Morphine hydrochloride, dissolved in Dulbecco's modified Eagle's medium, was added (50 μl) to 5 ml

of incubation medium. After the incubation, medium and cells were separately assayed for cyclic AMP^{23,30}. The data are referred to cellular protein³⁰.

Curve *a* of Fig 1 demonstrates that morphine at concentrations between 0.1 and 100 $\mu\text{mol l}^{-1}$ inhibits the increase of cyclic AMP levels evoked by PGE₁. Up to 12% of the total cyclic AMP thus formed is found in the incubation media (Fig 1, curve *b*). Release of cyclic AMP into the surrounding media has been described previously^{23,31}. As the fraction of cyclic AMP released from the cells is always low in these conditions, however, the corresponding data are omitted from the subsequent figures. The inhibitory action of morphine decreases at concentrations above 0.1 mmol l^{-1} (Fig 1, curve *a*). We observed²³ a qualitatively similar phenomenon using another antagonist of PGE₁, 7-oxa-13-prostanoic acid²⁴. It remains to be established whether or not the two phases of Fig 1, curve *a* correspond to the sites of stereospecific and non-stereospecific opiate binding³². In cells not stimulated by PGE₁, no effect of morphine on the level of cyclic AMP is noticed (Fig 1, curve *c*). Again, only about 10% of the total cyclic AMP is found in the medium.

On exposure to PGE₁ the level of cyclic AMP reached in neuroblastoma \times glioma hybrids is considerably higher than in neuroblastoma line N4TG3. The cells are more sensitive to morphine than N4TG3 (Fig 2, curve *a*). The 50% inhibitory concentration (IC₅₀) is 5 $\mu\text{mol l}^{-1}$ for the hybrids compared with 400 $\mu\text{mol l}^{-1}$ for the neuroblastoma line. In three further series of experiments with N4TG3, however, the IC₅₀ was in the range of 5–20 $\mu\text{mol l}^{-1}$. The causes for this scattering of morphine-sensitivity values are under investigation. IC₅₀ values of 5 $\mu\text{mol l}^{-1}$ were also reported for the adenyl cyclase system in brain homogenates²⁹. In considering the cell culture system as models for nervous tissues in intact animals, it is important that it is also micromolar concentrations of morphine in brain which exert an antinociceptive effect (data recalculated from ref 33). Similar to the neuroblastoma line, the hybrids show no sensitivity to morphine in the absence of PGE₁ (Fig 2, curve *b*).

The morphine analogue naloxone is known to antagonise the action of morphine without causing morphine-like effects itself³⁴. As seen in Fig 3, naloxone exerts such an effect also on

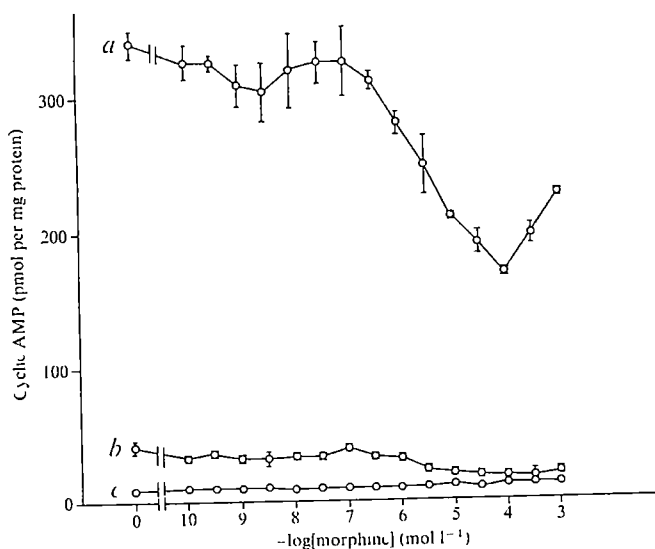


Fig 1 Inhibition by morphine of the stimulation by PGE₁ of cyclic AMP formation in neuroblastoma clone N4TG3. N4TG3 is a 6-thioguanine resistant mutant²⁰ of a clonal line derived from neuroblastoma C1300 (ref 3). At varying concentrations of morphine, the cells are incubated in the presence and absence of PGE₁. Subsequently, the cyclic AMP formed is measured. *s.d.* are given together with the mean value of three parallel incubations. Data without standard deviations are obtained from single plates. 17.6 $\times 10^6$ cells per plate, passage number 27, viability (exclusion of nigrosin) 96%. *a*, cyclic AMP in cells + medium, 10 $\mu\text{mol l}^{-1}$ PGE₁; *b*, cyclic AMP released into the incubation medium, 10 $\mu\text{mol l}^{-1}$ PGE₁; *c*, cyclic AMP in cells plus medium, no PGE₁.

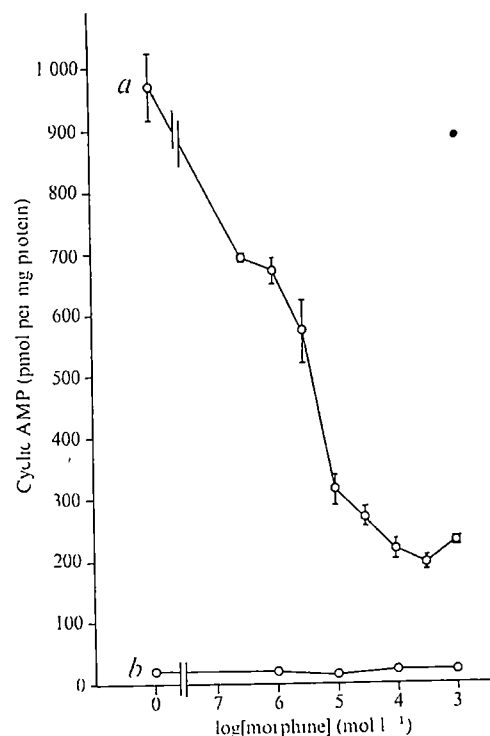


Fig 2 Inhibition by morphine of the stimulation by PGE₁ of cyclic AMP formation in clonal neuroblastoma N18TG2 \times glioma C6-BU-1 hybrid line 108CC15. A brief description of the cells has been given elsewhere^{18,20}, a detailed report is in preparation. Passage number 14, 5.9 $\times 10^6$ viable cells per plate, viability 85% cyclic AMP in cells, *a*, 3 $\mu\text{mol l}^{-1}$ PGE₁; *b*, no PGE₁.

neuroblastoma cells. The inhibitory effect of 0.1 mmol l^{-1} morphine is abolished by 1–10 $\mu\text{mol l}^{-1}$ naloxone (Fig 3, curves *a* and *b*). Furthermore, in concentrations above 0.1 mmol l^{-1} , naloxone itself causes an elevation of the level of cyclic AMP (Fig 3, curve *c*), and, in combination with PGE₁, potentiates the action of the latter (Fig 3, curve *a*). This effect is even greater when morphine is also present (Fig 3, curve *b*). This result suggests that in the concentration range of 0.1–1 mmol l^{-1} morphine and naloxone are not antagonists but rather act synergistically (Figs 1 and 3). Work is under way to elucidate this phenomenon. In Fig 3, the biphasic shape of curves *a* and *b*, together with the notion that naloxone and PGE₁ increase the levels of cyclic AMP well above that seen when PGE₁ is present alone, indicates two different binding sites for naloxone. The similarity of the effects exerted by morphine and naloxone at high concentrations and their synergism might suggest that the drugs act at the same low affinity binding sites³² and that the specificity of binding is relatively low.

This study adds another item to the increasing list of differentiated nervous functions found in cell lines derived from the nervous system and suggests the suitability of such cells as model system for the investigation of opiate action. In particular, the antagonism between PGE₁ and morphine in the cultured cells parallels strikingly the antagonism observed in intestinal contraction^{25,27,28}. Although in both N4TG3 and 108CC15 the increase of the intracellular levels of cyclic AMP is inhibited by morphine, the hybrid line is more promising as a model system. It is more sensitive to PGE₁ and to morphine than N4TG3. The inhibitory effect of morphine is non-competitive as it is not reversed at high PGE₁ concentrations (30 $\mu\text{mol l}^{-1}$). This is more than 300 times the dose at which PGE₁ elevates cyclic AMP to half maximal value³⁵. Furthermore, not all cell types sensitive to PGE₁ are also sensitive to morphine, as shown in a glioma and glioma \times fibroblast hybrid line³⁵. Like PGE₁, noradrenaline increases the level of cyclic AMP in these cells. Furthermore, this response is not inhibited by morphine. Thus, the antagonistic action of morphine may be restricted both with respect to the cell type and to the hormone affected.

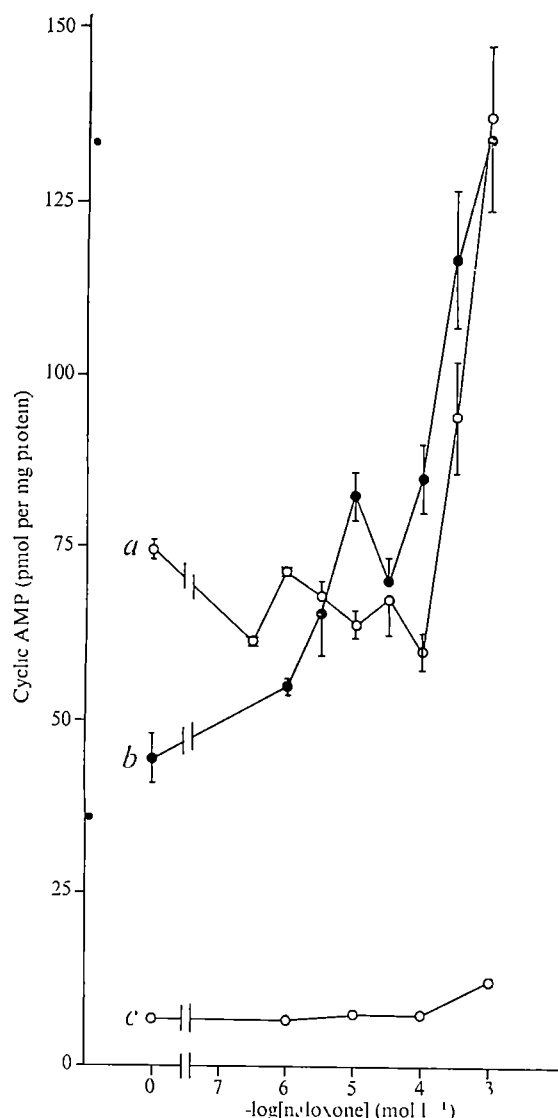


Fig. 3 Naloxone prevents the inhibition by morphine of the cyclic AMP formation stimulated by PGE_1 . 1.61×10^6 viable N4TG3 cells, passage number 29, viability 91%, $3 \mu\text{mol l}^{-1}$ PGE_1 , b , $3 \mu\text{mol l}^{-1}$ $\text{PGE}_1 + 0.1 \text{ mmol l}^{-1}$ morphine, c , without PGE_1 and morphine. The inhibitory effect of morphine in the absence of naloxone is evident, when the points at 0 mol l^{-1} naloxone of curves a and b are compared.

The study of the clonal cell lines sensitive to morphine may have provided a first glimpse at the mechanisms involved in opiate action. The advantage of the system is its simplicity. The cell masses of a clone are relatively homogeneous compared with the complex mixture of cells in brain. Roy and Collier²⁹ studying rat brain homogenates found an adenylyl cyclase which is stimulated by PGE_1 , the stimulation is inhibited by morphine. The use of the cell-free system may be limited by the high background activity of adenylyl cyclase in the absence of PGE_1 and by the very low sensitivity to PGE_1 . The rat brain homogenates are at least 300 times less sensitive to PGE_1 than N4TG3 cells^{29,35}. Nevertheless the major findings—the antagonism between PGE_1 and morphine and the involvement of the adenylyl cyclase system in morphine action—are the same with both approaches and thus the conclusions drawn are similar.

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Action potentials induced in slow muscle fibres by partial denervation

Frog slow muscle fibres are usually unable to generate action potentials^{1,2}. It has been demonstrated, however, that this ability is acquired after denervation^{3,4}, and it has been suggested that the inhibitory effect of the small motor axons on the slow fibre membrane results from the release of a 'trophic' substance⁴⁻⁶. Since slow muscle fibres are usually innervated by more than one motor axon⁴, it was important to find out, whether the action potential is induced after partial removal of this 'trophic' influence. Our results show that this is indeed the case.

The experiments were performed on the right pyriformis muscle of *Rana temporaria*. This muscle is usually innervated by spinal branch IX, it has, however, been observed⁷ that occasionally the muscle receives additional motor axons from spinal branch VIII. One of these branches, usually IX, was cut in the pelvis. After the operation the frogs were kept at room temperature (about 19°C), and 11–13 d later the pyriformis muscle was removed, together with its nerve, for

electrophysiological examination⁸. The slow fibres of the piriformis muscle are 10–15 mm long and their length constant is of the same order of magnitude⁹. With a single insertion of the microelectrode measuring potential (about half-way between the tendons), the whole fibre can therefore be explored. Rectangular current pulses were applied to a second microelectrode inserted at a distance of 50–100 μm and the slow fibres were identified by their electrical properties⁹. The nerve was mounted on two pairs of platinum electrodes for stimulation of motor axons and determination of their conduction velocities⁸. Ringer's solution had the following composition (mM): NaCl 110.4, KCl 2.5, CaCl_2 7.2, Tris 5.0. All measurements were performed at 8–10°C.

In all, sixteen muscles were examined after transection of spinal branch IX (14 muscles) or VIII (2 muscles), twelve muscles proved to be completely denervated, while in the remaining four muscles variable proportions of denervated muscle fibres were identified. The slow fibres could be subdivided into three groups. Three fibres (group 1) were completely denervated, presumably because they were originally innervated only by the spinal branch which had been cut, showed action potentials as expected (Fig 1A). Nine fibres (group 2) showed action potentials, generally of smaller amplitude but in contrast to group 1 they responded with an end-plate potential to nerve stimulation (Fig 1B). Three fibres (group 3) were also innervated but did not produce action potentials, and therefore seemed to be normal slow fibres.

Similar results were obtained in a second series of experiments, in which one small branch of the piriformis motor nerve was cut 1–2 mm from its entry into the muscle. Among the 43 slow fibres identified 8–15 d later 26 belonged to group 2, being innervated and yet capable of generating action potentials. Eight fibres were completely denervated and produced action potentials (group 1), while nine fibres seemed to be normal (group 3).

It is important to eliminate the possibility that those slow fibres (group 2) showing both end-plate and action potentials were in the early stage of reinnervation after having been completely denervated. Reinnervation by regenerated motor axons could be excluded, because inspection of the operation site revealed that the proximal and distal nerve stumps were always clearly separated, furthermore there would not have been enough time for regeneration, at least in those experi-

ments in which the spinal branch had been cut. The alternative possibility, that reinnervation of totally denervated fibres had taken place by collateral sprouting from intact axons also seems unlikely in view of the short period (sometimes only 8 d) that had elapsed after the operation. Furthermore the characteristics of the end-plate potentials from the group 2 fibres also suggested that they occurred at residual 'old' end-plates rather than newly formed ones. Thus their amplitudes were usually smaller (mean 18 mV) than normal (30 mV) but not markedly different, and they occurred with normal latencies. Moreover, the majority of fibres (33 out of 35) were innervated by a single slow axon (conduction velocity smaller than 5.2 m s^{-1}).

Our experiments demonstrate that two or more small motor axons are necessary to suppress the action potential mechanism in slow muscle fibres. It is conceivable that each individual axon exerts its effect locally as has been shown for the neural control of acetylcholine sensitivity in frog twitch fibres¹⁴. In this case the transformation of the slow fibre membrane could be restricted to the vicinity of degenerating end-plates, while the membrane remains unchanged near normal end-plates. It may also be that cutting one or more small motor axons leads to a general reduction of the inhibitory effect such that sodium-channels are simultaneously built into the membrane along the entire length of the fibre. At present we cannot distinguish between these two possibilities and more work is needed to understand how the small motor axons exert their 'trophic' effect on slow muscle fibres. It seems clear, however, that a reduction in size of the end-plate potential is not directly responsible because Botulinum toxin markedly reduces the end-plate potential without inducing an action potential¹⁵.

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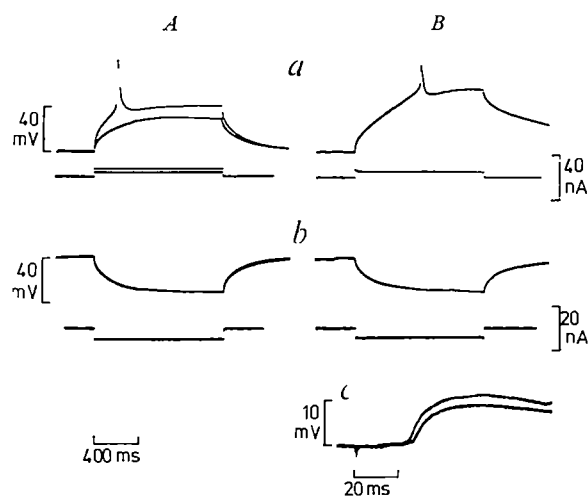


Fig 1 Action potentials (a) and anelectrotonic potentials (b) recorded from a denervated (A) and an innervated (B) slow fibre 9 days after cutting spinal nerve IX. Upper trace, membrane potential, lower traces, membrane currents. Note large membrane time constant typical of slow fibres. c, End-plate potentials elicited in innervated slow fibre by stimulation of spinal nerve VIII at two points 10 mm apart, from the difference in latencies a conduction velocity of 4.4 m s^{-1} was calculated for the motor axon. Both fibres from same muscle. Note also the presence of miniature end-plate potentials in trace b of fibre B.

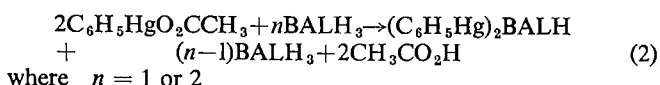
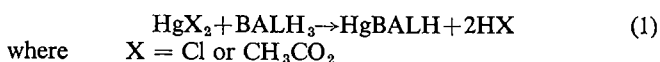
British anti-Lewisite and organomercury poisoning

2,3-DIMERCAPTOPROPANOL ($\text{CH}_2(\text{OH})\text{CH}(\text{SH})\text{CH}_2\text{SH}$, BALH₃) is generally used in the treatment of mercury poisoning to help remove mercury from the body^{1–3}. It has been reported, however, that use of BALH₃, together with, or shortly after injection into laboratory animals of inorganic mercury^{4–6}, methylmercury^{7–8}, or 'phenylmercury'^{7–9} compounds results in a different initial distribution of mercury in the body than when the compounds are injected alone. For inorganic mercury, the results can be explained in terms of the timing and dosage of BALH₃ (ref. 6). For both methyl and phenylmercury compounds with BALH₃, the mercury content of the brain is greatly enhanced^{7–9}. Although the initial rapid (24 h) distribution of mercury in the animals treated for methylmercury poisoning with BALH₃ is similar to that achieved after 8 d in the absence

of BALH_3 (ref 8), this distribution is undesirable in cases of chronic mercury poisoning where the critical organ is the brain, in which it is metabolised more slowly than in most organs^{10,11}

An understanding of the chemistry involved in the interaction of BALH_3 with mercury compounds, and of the properties of any complexes formed, is necessary to assess these observations. Isolation of $\text{HgBALH}^{1,12}$ has been reported and evidence for the formation of $\text{Hg}(\text{BALH}_2)^{2-}$ (ref 1), $(\text{C}_6\text{H}_5\text{Hg})_2\text{BALH}$ (ref 12) and $(\text{RHg})_n\text{BALH}_{3-n}$ ($n = 1$ (ref 13), 2 (ref 12), $\text{R} = \text{CH}_2\text{CH}(\text{OCH}_3)\text{CH}_2\text{R}'$) has been documented

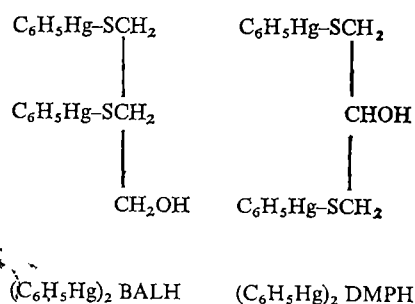
We have found that reaction of mercuric salts and phenylmercuric acetate with BALH_3 in water leads to precipitation of HgBALH and $(\text{C}_6\text{H}_5\text{Hg})_2\text{BALH}$, respectively



To assist interpretation of physical and spectroscopic data for the complexes (Table 1) we prepared the analogous 1,3-dimercaptopropanol(DMPH₃) complexes

All the complexes are insoluble in water and common organic solvents. Except for HgBALH they dissolve in the coordinating solvents pyridine and dimethylsulphoxide, this may indicate polymeric structures or strong intermolecular forces in the solid state. Assignment of $\nu(\text{Hg-S})$ and $\nu(\text{O-H})$ in infrared spectra of the complexes (Table 1) establishes the basic mode of attachment of mercury to BALH and the loss of two thiol protons on complex formation. Assignment of a coordination number for mercury is, however, not possible since $\nu(\text{Hg-S})$ does not vary greatly with coordination number in related compounds, for example, two-coordinate mercury in $\text{Hg}(\text{SC}_2\text{H}_5)_2$ (ref 14) has $\nu(\text{Hg-S})$ at 330 cm^{-1} , and four-coordinate mercury in *bis* (O-ethylthioacetothioacetato) mercury has $\nu(\text{Hg-S})$ at 362 cm^{-1} (ref 15)

HgDMPH is associated in pyridine (Table 1), consistent with a polymeric or oligomeric structure in the solid state, and HgBALH presumably adopts a similar structure. Molecular weight data indicate monomeric structures for the phenylmercury derivatives in pyridine



Since a variety of 'solvent' environments, for example, aqueous and lipid, are present in organisms, the stability of $(\text{C}_6\text{H}_5\text{Hg})_2\text{BALH}$ in different solvents is of particular interest. In aqueous suspension, $(\text{C}_6\text{H}_5\text{Hg})_2\text{BALH}$ is stable with an excess of BALH_3 since it can be prepared with $n = 2$ (equation (2)); and the possible decomposition products expected, $(\text{C}_6\text{H}_5)_2\text{Hg}$, HgBALH or benzene (gas liquid chromatography), could not be detected. Although stable in pyridine and dimethylsulphoxide (Table 1), addition of acetone to a pyridine solution results in precipitation of HgBALH and formation of diphenylmercury. When $(\text{C}_6\text{H}_5\text{Hg})_2\text{BALH}$ is stirred as a slurry in benzene for 19 h, the insoluble residue contains $(\text{C}_6\text{H}_5\text{Hg})_2\text{BALH}$ and HgBALH , $(\text{C}_6\text{H}_5)_2\text{Hg}$ can be isolated from the solution. Similarly, reaction of phenylmercuric acetate with BALH_3 (2 1) in methanol for 2 h gives a mixture

Table 1 Characterisation of 2,3-dimercaptopropanol (BALH_3) and 1,3-dimercaptopropanol (DMPH_3) complexes*

Complex	Infrared (cm^{-1})†		Molecular weight‡	
	$\nu(\text{Hg-S})$	$\nu(\text{O-H})$	Observed	Calculated for monomer
HgBALH	350	3,325	Insoluble	323
$(\text{C}_6\text{H}_5\text{Hg})_2\text{BALH}$	335	3,330	664 ± 15	678
HgDMPH	353	3,330	500 ± 67 §	323
$(\text{C}_6\text{H}_5\text{Hg})_2\text{DMPH}$	343	3,320	653 ± 43	678
$(\text{C}_6\text{H}_5\text{Hg})_2\text{DMPH} \cdot \text{C}_6\text{H}_5\text{N}¶$	343	—	750 ± 34	757
$\text{C}_6\text{H}_5\text{HgSCH}_2\text{CH}_2\text{OH} $	343	3,230	364	355
$\text{Hg}(\text{SCH}_2\text{CH}_2)_2 $	330	—	330	323

* All complexes have satisfactory elemental analyses ($\text{C}, \text{H}, \text{Hg}, \text{S}$) and, except for insoluble HgBALH , have satisfactory ^1H NMR spectra in both d^5 -pyridine and d^6 -DMSO

† Nujol mulls. All absorptions listed are broad. $\nu(\text{S-H})$ is absent from all spectra, indicating loss of two protons on formation of each complex

‡ In pyridine at 60°C determined by vapour pressure osmometry. Where several determinations were obtained the range is given. Since pyridine is a non-ideal solvent, accurate molecular weights are not expected. However, complex formation with pyridine (see for example ¶ below) as one form of non-ideal behaviour is not expected to affect the accuracy of determinations, since the colligative property determined is the number of molecules formed in solution per solid complex added, and this will not be altered by interaction with the solvent which is present in vast excess

§ These results are intermediate between monomer and dimer, indicating the presence of a monomer-oligomer(s) (most likely dimer) equilibrium. In addition NMR spectra have a very broad methylene resonance, suggesting more than one methylene environment

¶ $(\text{C}_6\text{H}_5\text{Hg})_2\text{DMPH}$ forms a crystalline 1:1 adduct with pyridine. An unaltered $\nu(\text{Hg-S})$ and lowered $\nu(\text{O-H})$ (very broad absorption beneath $\nu(\text{C-H})$ bands at $2,800\text{--}3,200\text{ cm}^{-1}$) indicate that pyridine is hydrogen-bonded to the hydroxyl group, rather than coordinated to mercury. With the hydroxyl group deuterated $\nu(\text{O-D})$ occurs at $2,200\text{ cm}^{-1}$. The calculated molecular weight is that expected for dissolution of a 1:1 mixture of $(\text{C}_6\text{H}_5\text{Hg})_2\text{DMPH}$ and pyridine in pyridine

|| Included for comparison

of $(\text{C}_6\text{H}_5\text{Hg})_2\text{BALH}$, HgBALH and $(\text{C}_6\text{H}_5)_2\text{Hg}$. Thus, with some solvents, $(\text{C}_6\text{H}_5\text{Hg})_2\text{BALH}$ decomposes according to



The formation of diphenylmercury had not previously been suspected and may be an important factor in the distribution of mercury when BALH_3 is administered with phenylmercuric acetate. Diphenylmercury is less polar than Hg^{2+} and $\text{C}_6\text{H}_5\text{Hg}^+$, does not form complexes with neutral ligands¹⁶ and does not react with thiols^{17,18} or BALH_3 at ambient temperature. The toxicity of diphenylmercury does not seem to have been investigated, except for a brief report¹⁹ that diphenylmercury in 'scarcely detectable' concentration, formed by degradation of phenylmercuric acetate (formerly contained in derelict steel drums), was sufficiently toxic to kill fish within a few hours in the Boone Reservoir, Tennessee Valley.

These preliminary results indicate the complexity of antidote action and the need for caution in application of BALH_3 as an antidote for chronic phenylmercury poisoning. This is particularly true before degradation to inorganic mercury, which is substantially complete after 1 d in laboratory animals²⁰⁻²². This study is being extended to include alkylmercury compounds.

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Effect of unsaturated fatty acids on the lipid composition of bacteriophage PM2

A LIPID-containing bacteriophage, PM2, provides a simple model to investigate assembly of lipids and proteins into a membrane. The phospholipid in the virion, which is arranged in the form of a bilayer between nucleoprotein core and outer shell¹, has a very different composition from that of the host cell, a marine bacterium, *Pseudomonas* BAL-31 (ref 2). One-third of the viral lipids are derived from lipids synthesised before infection and two-thirds from lipids synthesised during viral replication³.

We have studied the variation in viral fatty acids and phospholipids when the virus was grown in an auxotroph

requiring unsaturated fatty acids, *Pseudomonas* BAL-31, strain UFA (ref 4). The fatty acid composition in host cells and virions varied in a similar manner in media supplemented with different fatty acids, whereas the phospholipid composition of the host cell was almost constant, that of the virion varied depending on the fatty acids used in the media.

Effects of exogenous unsaturated fatty acids on the viral lipid composition were determined using *Pseudomonas* BAL-31, UFA as host. The fatty acid composition in host cells and virions grown in media supplemented with unsaturated fatty acids is shown in Table 1. In media supplemented with palmitoleic (*cis*-16:1) and oleic (*cis*-18:1) acids, the fatty acid composition of phospholipids in virions and host cells seemed to be identical, within experimental error. In medium supplemented with palmitelaidic acid (*trans*-16:1), the amount of palmitic acid (16:0) in virions was larger than that in host cells and the amount of 16:1 in virions was smaller. Virions grown in media supplemented with *cis*-16:1 and *trans*-16:1 had nearly homogenous fatty acid compositions, 16:1 accounting for 89% and 74% of the total fatty acids, respectively. In general, the fatty acid composition in virions reflects that in the host cells and thus can be altered by variation in the exogenous fatty acid.

The phospholipid composition in host cells and virions grown in media supplemented with unsaturated fatty acids is shown in Table 2. In all media except that supplemented with *trans*-16:1, the major phospholipid in virions was phosphatidylglycerol (PG). In medium with *trans*-16:1, phosphatidylethanolamine (PE) became the dominant viral phospholipid. The ratio of PG to PE in viral lipids varied with the input fatty acid. In virions grown on *Pseudomonas* BAL-31 (wild type) in a medium without added fatty acid it was about 1.5, in virions grown on strain UFA in media supplemented with *cis*-16:1 or *cis*-18:1 it was about 1.2, and in virions grown on strain UFA in *trans*-16:1 medium about 0.8. In earlier studies^{2,5} the PG/PE ratio in virus grown on wild type cells in a medium containing the same salts as used above, plus arginine, proline and glucose, was about 2.4. This discrepancy may be the result of differences in the medium used. In contrast to the situation with fatty acids and phospholipids, virions purified from strain UFA grown in the presence of different fatty acids had the same pattern of polypeptides (using SDS-polyacrylamide gel electrophoresis) as those prepared from wild type *Pseudomonas* BAL-31.

Table 1 Fatty acid composition of phospholipids in *Pseudomonas* BAL-31/UFA and PM2 grown in media supplemented with unsaturated fatty acids

Fatty acids in phospholipid	Unsaturated fatty acid added to growth medium					
	Palmitoleate (<i>cis</i> -16:1)		Palmitelaidate (<i>trans</i> -16:1)		Oleate (<i>cis</i> -18:1)	
	UFA	PM2	UFA	PM2	UFA	PM2
14:0	—	—	trace	trace	0.5	0.4
14:1	0.6	2.0	10.7	10.0	1.4	1.9
16:0	3.0	4.7	0.7	7.6	7.2	4.7
16:1	88.8	89.0	86.1	74.2	57.2	58.8
18:0	0.6	0.3	0.2	3.4	0.9	0.7
18:1	7.0	4.0	2.3	5.0	32.8	33.5
Saturated	3.6	5.0	0.9	11.0	8.6	5.8
Unsaturated	96.4	95.0	99.1	89.0	91.4	94.2
Unknown	trace	trace	trace	trace	trace	—

For the isolation of fatty acid auxotrophs, *Pseudomonas* BAL-31 was grown at 25°C in AMS salts¹² supplemented with 2 g l⁻¹ of the amino-acid mixture containing 0.1 g l⁻¹ of twenty amino acids (AA medium) and 0.01% oleic acid dissolved in 0.4% bovine serum albumin containing less than 0.05% fatty acids. After ultraviolet irradiation to the 0.5% survival level, fatty acid auxotrophs were isolated using penicillin enrichment in AA medium and replica plating on selective media. A culture (25 ml) of *Pseudomonas* BAL-31 fatty acid auxotroph strain UFA grown in the presence of different unsaturated fatty acids was infected (multiplicity of infection 10) with PM2 which had been grown on wild type cells. This lysate was used to infect 100 ml cultures, which were used in turn for 1 l of culture. Virus was purified from 1 l lysate 3 h after infection⁶. The purity of each virus preparation was controlled by SDS-polyacrylamide gel electrophoresis¹³. Lipids were extracted from purified virus and from host cells which were collected just before infection³. Phospholipids were then isolated on silica gel thin layer plates (Merck, *F₂₅₄*) using diethylether-benzene-ethanol-acetic acid (40:50:2:0.2, v/v). The area corresponding to the phospholipids was made visible with iodine vapour, then scraped and eluted with chloroform-methanol (2:1, v/v). Phospholipid was trans-methylated with BF₃-methanol (14%, w/v, Applied Science Laboratories). Methyl esters were analysed on a Packard model 7620A gas chromatograph with 10% EGSSX on Chromosorb W and 3% SE30 on Gas Chrom Q. Methyl esters were identified by comparison of their retention times with those from a KD ester mixture (Applied Science Laboratories). Results are expressed as percentage composition.

Table 2 Phospholipid composition in *Pseudomonas* BAL-31/UFA and PM2 grown in media supplemented with unsaturated fatty acids

Phospholipid	Palmitoleate (<i>cis</i> -16:1)		Unsaturated fatty acid added to growth medium Palmitelaidate (<i>trans</i> -16:1)		Oleate (<i>cis</i> -18:1)		None	
	UFA	PM2	UFA	PM2	UFA	PM2	BAL-31	PM2
Compound X	0.2	1.8	3.8	6.9	0.1	0.8	0.1	0.6
	0.1	1.0	2.7	1.2	0.1	1.0	0.4	0.6
Phosphatidyl-ethanolamine	78.8	41.7	82.3	49.6	80.8	47.0	75.8	39.5
	85.6	43.7	84.8	53.8	89.1	41.2	80.5	38.4
Phosphatidyl-glycerol	18.9	53.3	12.1	41.1	17.4	50.0	22.0	58.2
	13.2	52.5	11.0	42.0	10.2	55.8	17.9	59.4
Lysophosphatidyl-ethanolamine	2.0	1.7	1.7	1.2	1.3	1.4	1.6	0.9
	0.4	1.8	1.0	2.1	0.4	1.4	0.6	1.0
Others	0.1	1.6	0.1	1.2	0.4	1.1	0.5	0.9
	0.6	1.1	0.6	0.9	0.2	1.0	0.6	0.7
Total radioactivity (c.p.m.)	97,019	102,381	83,500	73,474	74,491	65,926	65,002	99,916
	82,910	28,604	65,143	28,458	61,135	25,686	59,417	57,324
Ratio PG/PE	0.24	1.28	0.15	0.83	0.22	1.06	0.29	1.47
	0.15	1.20	0.13	0.78	0.12	1.36	0.22	1.55

Pseudomonas BAL-31 and strain UFA were grown at 25° C in AA medium with 0.4% bovine serum albumin and 0.01% essential fatty acid. The culture (25 ml) was lysed by PM2 grown on wild type cells. This lysate was used in turn for lysing 100 ml culture containing 3 μ Ci ml⁻¹ ³²P. Virus was purified along with carrier virus grown on wild type cells and its purity was controlled by SDS-polyacrylamide gel electrophoresis, as described in Table 1. Lipids were extracted from virus and host cells, which were withdrawn just before infection as described in Table 1. Lipids were analysed on silica gel thin layer plates (Merck, F₂₅₄) using chloroform-methanol-water (65:25:4, v/v). Each class of phospholipids was detected by exposure to Ilford X-ray film, scraped into vials and counted for radioactivity². Results are expressed as percentage composition.

There are four viral proteins I and II form spikes and outer shell, respectively, III forms the inner shell of the nucleocapsid, while IV is associated with viral DNA (ref. 6). Proteins I, III and IV have isoelectric points at pH 6.2, 5.8 and 5.5, respectively, whereas the isoelectric point of protein II is at pH 12.3. Thus protein II is strongly positively charged at pH 7.0 (refs 7 and 8). The electrostatic interaction between protein II and PG has been suggested as a major factor controlling phospholipid composition and also stabilising the bilayer structure of the completed particle^{7,8}. The PG/PE ratio would remain constant, provided that the fatty acids do not affect the charge distribution and that long range forces play only a minor role. In addition to electrostatic forces, there is evidence for hydrophobic interactions between the bilayer and the inner protein shell as protein III behaves as a proteolipid^{8,9}, and some viral protein(s) seems to penetrate the lipid bilayer and may also participate in lipid-protein interactions.

The physical properties of phospholipids containing different hydrocarbon side chains have been studied using monolayer techniques¹⁰. *Trans*-unsaturated fatty acids have properties intermediate between *cis*-unsaturated fatty acids and saturated fatty acids, forming more condensed films than *cis*-unsaturated fatty acids. Virions grown on strain UFA in the presence of exogenous *trans*-16:1 should therefore have the most condensed lipid bilayer of those reported here, which should lead to a stronger hydrophobic interaction between proteins and lipids within the membrane structure and may thereby overcome the electrostatic interaction. Furthermore, if we compare the known mobility of PE and PG (ref. 11) with the phospholipid composition of virions containing *trans*-16:1, the average fluidity must have decreased. Thus the change in phospholipid composition can hardly be due to a tendency to maintain average fluidity. Although the phospholipid composition in virions varies according to the fatty acid used in the medium, such variations are controlled within narrow limits. Thus both hydrophobic and electrostatic interactions between lipids and proteins probably assist in maintaining viral integrity.

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The double helix of tropomyosin

THE tropomyosin molecule consists of two α helices, which form a coiled-coil a little over 400 Å long¹. Besides forming crystals, tropomyosin can be precipitated with divalent cations to produce needle shaped aggregates (showing a variety of patterns of cross striations in the electron microscope²) for which the detailed molecular packings have not been established. At low resolution, however, the α helices may be regarded as smooth rods. The structure of such aggregates could depend, to some extent, on considerations of closest packing of the rods. This possibility seems to have been first considered by Rudall³ in his discussion of the crystal structure of the α protein from Mantis egg case, where he showed that double helices may be close-packed to form sheets by staggering each double helix by one quarter of the pitch with respect to the next. I report here that the idea of a quarter stagger between tropomyosin molecules, in conjunction with certain electron microscope results, leads to a value for the pitch of the tropomyosin helix.

Figure 1a shows an electron micrograph of vertebrate tropomyosin precipitated⁴ with CaCl₂ at pH 8, displaying sets

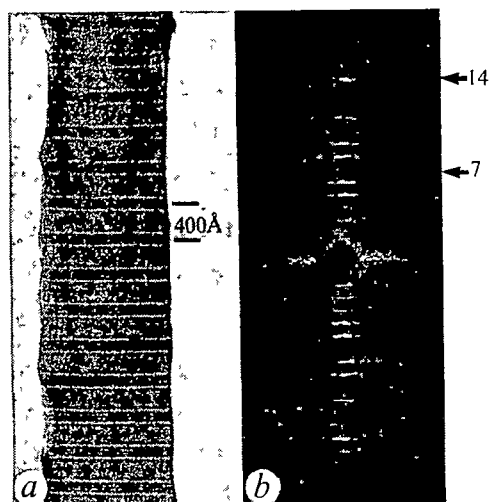


Fig. 1 *a*, Rabbit striated muscle tropomyosin precipitated with 20mM CaCl_2 at pH 8 *b*, Optical diffraction pattern of (*a*)

of transverse striations repeating axially every 400 Å. A striking feature of the optical diffraction pattern of this form is a prominent 14th order of the 400 Å repeat while the 7th order is weak or absent (Fig 1*b*). This suggests that there is a component in the structure which repeats every 57 Å but which is staggered in alternate units by one half of this distance. This suggestion has also been made by Parry and Squire⁵ who noticed that the electron micrographs of this form of tropomyosin show subperiods of about 28 Å and that "the separation of bands related by twofold symmetry is always an odd multiple of 28 Å".

These observations lead to the hypothesis that the pitch of the double helix in tropomyosin is 114 Å, in which case the repeat distance in the molecule would be 57 Å (the half-pitch), close packing would halve this to give a repeat in the sheets of 28.5 Å. The sheets could then superimpose, forming the observed structures. (The various ways in which such sheets can pack together to form three dimensional structures will be discussed elsewhere.) Accumulation of negative stain between the helices would then produce the subperiods observed with the electron microscope.

In the living muscle, tropomyosin is associated with actin which consists of two strands of subunits repeating along a helix with pitch of about 700 Å. Tropomyosin molecules lie in the two grooves, one on each side of the actin helix, and each molecule appears to interact with one strand. The vertical distance between the subunits of actin is about 55 Å. The distance along the helix between the subunits at the radius at which tropomyosin lies, is, however, close to 57 Å. (These findings have been summed up by Huxley⁶.) Thus, if the pitch of the tropomyosin double helix were 114 Å, each actin subunit would be in a position to interact with one half-turn of this helix. In this case, the chemical sequence of tropomyosin would be expected to display a similar periodicity, since each half-turn would have a similar chemical function to perform. An indication of such periodicity has recently been pointed out by Parry⁷ for the partial sequence of tropomyosin published by Hodges *et al.*⁸ Parry observed a pseudo-repeat of about 29 Å in the various types of residue groupings along the chain, so that each successive pair of these groups would lie opposite one actin subunit. Only if the pitch of the tropomyosin double helix were commensurate with the actin periodicity, however, could the repeating groups face towards the actin subunits to present to each a similar aspect.

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Reaction of ribonucleosides and deoxynucleosides with copper(II) acetate

BERGER *et al.* reported¹ that ribonucleosides and deoxynucleosides can be differentiated by their effects on the optical spectra of copper(II) acetate in dimethylsulphoxide (DMSO). They suggested that the 2' and 3' oxygen atoms in ribonucleosides bridge pairs of copper atoms, forming 1:1 copper(II) acetate/ribonucleoside complexes.¹ We have examined the species in such solutions by EPR. Optical spectra are not ideal as the bands are broad, and components of mixtures cannot readily be resolved. EPR spectra of spin-coupled ($S = 1$) copper(II) dimers differ appreciably, however, from those of monomeric ($S = \frac{1}{2}$) compounds. Moreover, they are sensitive to the nature of the bridges²⁻⁵ and to the symmetry about the Cu-Cu axis. Our conclusions from these studies differ from those obtained previously.¹

The X-band spectra of frozen DMSO solutions of copper(II) acetate alone and with various ribonucleosides and deoxynucleosides are shown in Fig 1. As in previous work¹ fresh solutions were used (10^{-2} M in copper(II) acetate). The $S = 1$ bands at about 40, 460 and 590 mtesla (mT) (Fig 1*a*) confirm that copper(II) acetate is dimeric in DMSO. The spectrum of a 1:1 copper(II) acetate dimer/uridine solution (Fig 1*b*) shows that uridine cleaves the dimer forming a large amount of a monomeric copper(II) species, with a strong signal at about 300 mT. There is no evidence for a dimer containing ribonucleoside bridges, which would give new $S = 1$ bands. Moreover, the presence of both acetate and ribonucleoside bridges would result in $E \neq 0$ and a split H_{xy} band.⁶ Instead, the residual $S = 1$ spectrum is that of copper(II) acetate.

The spectrum of copper(II) acetate and deoxyuridine (Fig 1*c*) shows that, in contrast to conclusions drawn¹ from optical spectra, the deoxynucleoside also cleaves the dimer, but to a lesser extent than uridine under the same experimental conditions. Deoxythymidine gave similar results. Both cytidine and deoxycytidine cause appreciable rupture of the dimer (Fig 1*d*). Deoxycytidine is more effective in this respect than deoxyuridine or deoxythymidine. The spectrum with cytidine differs significantly from that with uridine in the g_{\parallel} region of the monomer. Whereas the monomers formed by uridine, deoxyuridine and deoxythymidine each show one set of g_{\parallel} bands (Fig 1*e*), with cytidine (Fig 1*f*) there are two sets of g_{\parallel} components ($A, g_{\parallel} 2.304, A_{\parallel} 15 \text{ mT}$, $B, g_{\parallel} 2.249, A_{\parallel} 17.5 \text{ mT}$) indicating the presence of two monomeric species.

These results suggest that differentiation between ribonucleosides and deoxynucleosides by copper(II) acetate does not result from bridging by the 2' and 3' OH groups, but from the relative ease with which the dimeric structure is broken. Assuming complete protonation of N(3) in deoxyuridine and uridine, the major donor sites will be the sugar hydroxyl groups. Coordination of the 2' or 3' OH group of uridine to the terminal position of copper(II) acetate favours formation of a chelate ring by displacement of a neighbouring carboxylate oxygen by the other OH group of the ribonucleoside. Rupture of one acetate bridge would be followed by complete break-up of the dimer. As this mechanism is not available to deoxyuridine or deoxythymidine a differentiation between ribonucleoside and deoxynucleoside becomes possible.

We have observed, however, that deoxynucleosides also cause some rupture of the dimer. A possible reason is suggested by the results obtained with cytidine and deoxycytidine. Coordination of the non-protonated nitrogen, N(3), in cytidine

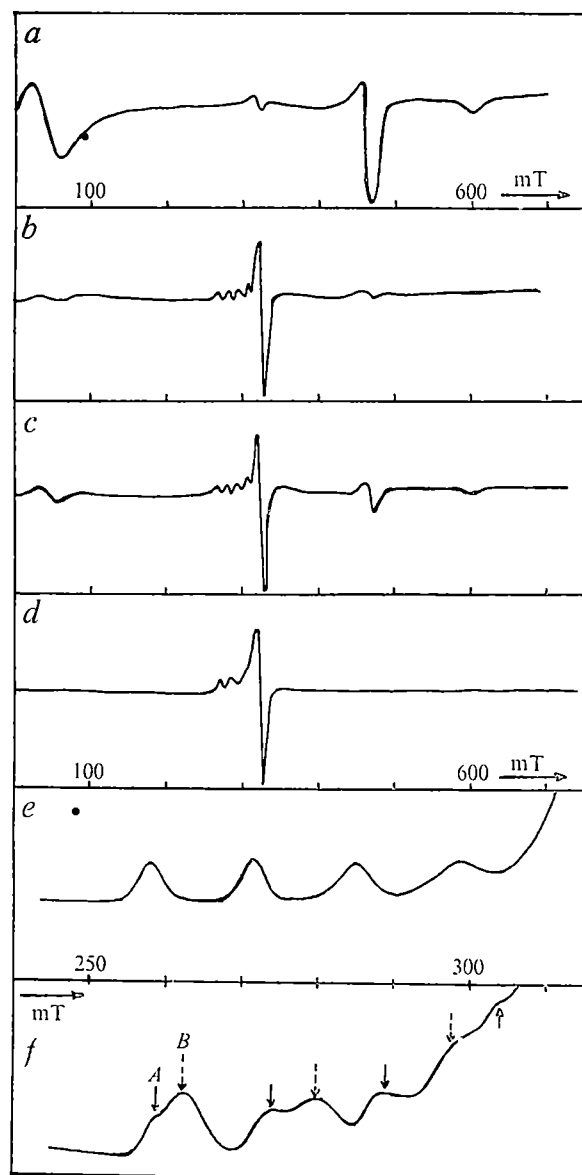


Fig. 1 X-band EPR spectra of frozen DMSO solutions of *a*, copper(II) acetate, *b*, copper(II) acetate and uridine (1 dimer 1 nucleoside), *c*, copper(II) acetate and deoxyuridine (1 dimer 1 nucleoside), *d*, copper(II) acetate and cytidine (1 dimer 1 nucleoside), *e*, as *b*, but showing the g_{11} bands of the $S=\frac{1}{2}$ spectrum in more detail, *f*, as *d*, but showing the g_{11} bands of the $S=\frac{1}{2}$ spectrum in more detail

and deoxycytidine to the terminal position in copper(II) acetate would bring the oxygen on C(2) and the amine group on C(4) close to acetate oxygen atoms and thus destabilise the dimer. With deoxycytidine this would be the main mechanism as there is no 2' OH group. With cytidine, however, either mechanism, that is, chelation by 2' and 3' OH groups or coordination by N(3), could operate. This would account for the two monomeric species observed. In polar solvents the N-H proton in deoxyuridine and deoxythymidine could undergo keto-enol tautomerism. Copper(II) acetate would displace this equilibrium towards the enol form by coordinating the non-protonated N(3) atoms. This would enable deoxyuridine and deoxythymidine to interact with copper(II) acetate, though to a smaller extent than deoxycytidine, as observed.

We conclude that copper(II) acetate does not 'recognise' the difference between ribonucleosides and deoxynucleosides by serving as a template for ribonucleoside bridges. Instead, the factors involve competing influences of the various co-

ordinating groups present, and in a broader biological context these influences will vary with the metal ion^{7,8}.

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Statistical significance of DNA sequence symmetries

SOME regions of DNA involved in regulatory or catalytic interactions with proteins have sites with twofold rotation symmetry in the primary nucleotide sequence¹⁻³. It would be worthwhile to determine whether certain of these symmetries occur so rarely by chance that they probably have a function, for example, as recognition sites for symmetric protein complexes. Likewise, it would be useful to be able to dismiss other apparent symmetries as probable statistical accidents of no functional importance. The following quantitative method can be used to evaluate the statistical significance of hyphenated (that is, incomplete) symmetries, where a more intuitive approach may be misleading. Since the probability of random occurrence of a given subsequence increases in almost direct proportion to the length of the sequence considered, a rigorous approach will become increasingly important in the evaluation of the significance of subsequence anomalies occurring in the much longer DNA sequences that will be determined in the future.

Rotation symmetry is said to exist in a DNA site if rotation about a twofold axis at the centre of the site and perpendicular to the DNA helix axis does not change the structure of the site. Various rotationally symmetric sites in bacteriophage λ DNA in the region of the joined cohesive ends (*cos*) and the region of repressor binding are indicated in Fig. 1. These symmetries are imperfect, and not all positions in the sites are invariant under rotation. The base pairs in these sites to be evaluated for symmetry have been marked *s*, *p* or *h* to designate whether under the rotation symmetry transformation they remain invariant (*s*), change but retain the same purine-pyrimidine orientation (*p*), or do neither of the above (*h*). The λ *cos* site designated in Fig. 1a has five perfect symmetries (*s*), five purine-pyrimidine symmetries (*p*), and one hyphen (*h*). A site with this extent of symmetry will be designated by the notation ($S_5P_5H_1$). Obviously, there is some probability that this symmetry could have arisen entirely by statistical accident. In a random sequence of 50% GC base composition, the *a priori* probability of an isolated occurrence of an *s* is 1/4, of a *p* is 1/4, and of an *h* is 1/2, so the *a priori* probability of the symmetry ($S_5P_5H_1$) in a sequence of length 2 ($S+P+H$) is

$$\frac{(S+P+H)^1 (1/4)^5 (1/4)^5 (1/2)^1}{S^1 P^1 H^1}$$

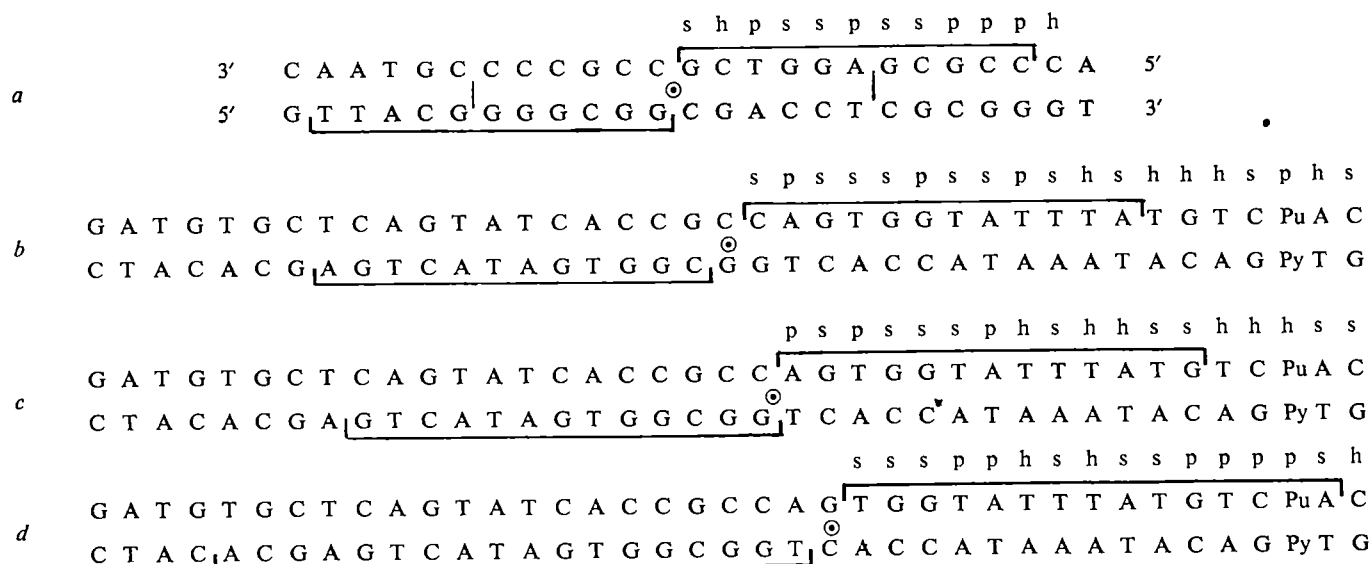


Fig 1 Symmetrical sites in certain regions of λ DNA. Bracketing lines indicate the two halves of a site symmetrical about a twofold rotation axis positioned at the symbol showing a dot within a circle. The symbol | designates the positions in λ *cos* at which single strand nicks must be introduced to generate cohesive ends. Other symbols are explained in the text: *a*, λ *cos* region (region of joined cohesive ends)³⁻⁵, 5_5s_{p1h} symmetry site, *b*, region of λ repressor binding¹, 8_3s_{p1h} symmetry site, *c*, region of λ repressor binding¹, 7_3s_{p3h} symmetry site, *d*, region of λ repressor binding¹, 7_6s_{p2h} symmetry site

The factorial coefficient arises because the order in which the various symmetries would occur in the site was not specified beforehand, so all significant permutations must be counted.

When a region of DNA is examined for sequence symmetries, the intention is usually to note all sites with sufficient symmetry to be of interest, rather than only sites of one specified degree of symmetry. Hence a satisfactory criterion for regarding an observed sequence to be exceptionally symmetrical is provided, not by the probability of occurrence of the symmetry, but rather by the probability that a random sequence of equal length would have an equal or greater symmetry.

The *a priori* probability, E_p , that at least M out of N positions in a site are either purine-pyrimidine symmetrical (p) or perfectly symmetrical (s) is

$$E_p = \sum_{i=M}^N \sum_{j=0}^i \frac{N! (1/4)^j (1/4)^{i-j} (1/2)^{N-i}}{j! (i-j)! (N-i)!}$$

$$= (1/2)^N \sum_{i=M}^N \sum_{j=0}^i \frac{N! (1/2)^i}{j! (i-j)! (N-i)!}$$

which is obtained by summing the individual probabilities of all such sites. Similarly, the *a priori* probability, E_s , that at least M positions in a site of length N are purine-pyrimidine symmetrical or perfectly symmetrical, and of these at least R positions are perfectly symmetrical is

$$E_s = (1/2)^N \sum_{i=M}^N \sum_{j=R}^i \frac{N! (1/2)^i}{j! (i-j)! (N-i)!}$$

The latter quantity, E_s , is of greater interest because it can be assumed that perfect symmetry will be more important than purine-pyrimidine orientation symmetry in most DNA recognition schemes, but the expectation of the symmetry can be shown to increase to E_p when the base composition of the region investigated is biased, for independent reasons, very heavily towards either GC or AT. For evaluation of an $(S_pP_pH_h)$ symmetry, the appropriate values of M , R and N in the above expressions are given by $M = S+P$, $R = S$, $N = S+P+H$. The expressions are most conveniently evaluated by computer.

The values of E_p and E_s for the sites shown in Fig 1 are tabulated in columns 3 and 4 of Table 1. These values reveal

Table 1 Symmetry and statistical significance of sites shown in Fig 1

Site illustrated	Rotation symmetry type	E_p , per-site probability of greater or equal purine-pyrimidine symmetry	E_s , per-site probability of greater or equal total symmetry	No. of sites of similar length in region considered	Expected frequency of sites of greater or equal total symmetry in region considered
Fig 1a	5_5s_{p1h}	5.86×10^{-3} = 1/171	3.70×10^{-3} = 1/270	—	—
Fig 1b	8_3s_{p1h}	3.17×10^{-3} = 1/315	3.79×10^{-4} = 1/2637	19	0.007
Fig 1c	7_3s_{p3h}	4.61×10^{-2} = 1/22	9.29×10^{-3} = 1/108	19	0.177
Fig 1d	7_6s_{p2h}	3.69×10^{-3} = 1/270	1.90×10^{-3} = 1/526	11	0.021

Column 2 indicates the symmetry type of the site according to the notation introduced in the text. Columns 3 and 4 give the rarity of occurrence of at least this degree of symmetry in a single site. Column 5 gives the number of such sites that can be tested for symmetry in the region under consideration, and the value in column 6, the product of values in columns 4 and 5, gives the expected frequency of occurrence of a site showing at least this degree of symmetry in the whole region under consideration.

that only about one sequence in 200 of length 22 would show at least the $(5_s, 5_p, 1_h)$ symmetry observed in the λ *cos* site. It would seem likely that this symmetry reflects some underlying mechanism. The significances of the symmetries at sites in the repressor-binding region of λ DNA vary considerably. When considered alone, these sites all appear to be quite rare, but this is misleading. If λ cohesive ends are generated by a symmetrical recognition-catalysis complex, the symmetry axis should, as it does, lie equidistant from the two sites at which nicks are to be introduced (Fig 1a). There is no such *a priori* basis for site selection, however, in the case of the λ repressor-binding region. Symmetries were considered wherever found. If the symmetry axis lies either on a base pair (as in Fig 1b) or between two base pairs (as in Fig 1c), then the number of sites comprised of two sequences of length N separated by zero or one base pair which can be tested for symmetry in a total sequence of length L is given by $2L-4N+1$. When each per-site expectation of symmetry is multiplied by the number of available sites, the results (Table 1, column 6) reveal that sites having at least $(8_s, 3_p, 1_h)$ symmetry, or $(7_s, 6_p, 2_h)$ symmetry, would be expected to occur in regions of 35 base pairs with a frequency of 0.7%, or 2%, respectively, but that sites having at least $(7_s, 3_p, 3_h)$ symmetry would be expected to occur with an 18% frequency. Hence the symmetries shown in Fig 1b and d would seem likely to be functionally significant, but the symmetry of Fig 1c could well be due to chance.

It can be shown that in a random DNA sequence the occurrence of symmetry about one rotation axis does not affect the probability of symmetry about a second, distinct axis (my unpublished work). Two sites symmetrical about different axes can therefore be considered independently, even if the sites overlap. This independence does not generally hold for more than two overlapping sites.

The lack of statistical significance in the observation of a symmetry does not necessarily imply that the symmetry is not functionally significant. Trial calculations reveal that it may be impossible to distinguish statistically certain functional symmetries from spurious symmetries unless the region under consideration can be restricted independently to considerably fewer than 100 base pairs.

This analysis determines the position of an observed symmetrical site in the symmetry-ordered distribution of only those sites which are of the same length. Although this comparison is limited, it provides an easily-computed estimate of the rarity of the symmetry. A similar approach could be used in the statistical evaluation of other types of sequence anomalies such as purine-pyrimidine orientation bias.

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Single-stranded regions in DNA of old mice

INVESTIGATIONS have shown that senescence is accompanied by molecular changes in the structure of the eukaryotic genome. Some of these changes have been reported to result in the loss of some genetic material in the brain cells of ageing beagles¹. Furthermore, the molecular weight of rat liver DNA decreases as a function of senescence². If such changes occurred on a large scale, the resulting genetic damage could affect severely some of the crucial gene functions of an organism. We are

studying the physical state of the DNA of senescing mice, and report here the accumulation of an increasing amount of single-stranded regions in the DNA with age.

DNA was isolated from the livers of 1, 6, 15, 20, 25, and 30-month-old CBF₁ mice (Charles River Breeding Laboratories). Single-strand-specific nuclease *S*₁ was used to investigate the presence of single-stranded regions in the native DNA of mice of different ages. Figure 1 shows the absence of nuclease *S*₁ sensitivity in the DNA of mice aged 1-15 months. That of mice 20 months of age or older was increasingly sensitive to the enzyme, which digested 14-25% of it. We cannot rule out the possibility that this sensitivity began before the mice were 20 months old, since we did not test the DNA of 15-20-month-old mice. These results are based on three experiments in each of which the DNA from different animals of the same age group were used.

There are already two sets of indirect evidence for single-stranded regions in the DNA of ageing animals. Samis *et al*³ reported increased incorporation of ³H-thymidine into the nuclear DNA of senescent rats in the absence of a corresponding increase in mitotic index. This implies that the thymidine, after conversion to dTTP, was used in the repair of nuclear DNA. Calf thymus DNA polymerase has been shown to catalyse a greater incorporation of DNA precursors into the nuclei of old mouse neurones, astrocytes, Kupffer cells and heart muscle fibres⁴. This reaction is known to require single-stranded regions of primer DNA along which the complementary strand can be synthesised⁵. Presumably these single-stranded regions of the DNA from senescent mice were hydrolysed by nuclease *S*₁ in our experiments.

As we do not know the ultimate fate of the nuclease *S*₁-sensitive regions in the DNA of old mouse liver, we suggest that

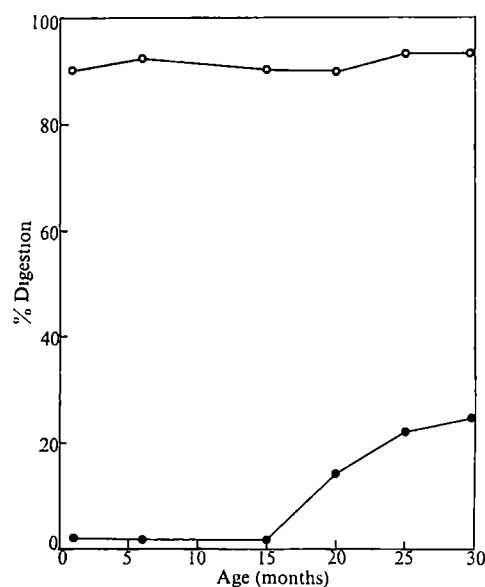


Fig. 1 Nuclease *S*₁ digestion of 1-30-month-old mouse liver DNA. DNA was prepared essentially by the method of Marmur³ except that a phenol-chloroform (1:1) mixture was used for extracting the proteins. Nuclease *S*₁ was prepared from *Aspergillus oryzae* crude α -amylase (Sigma) by the method of Sutton⁴. The reaction mixture consisted of 30 μ g of native or heat-denatured DNA, and 10 μ g nuclease *S*₁ all in 1.5 ml of KZS (0.1 M KCl, 0.1 mM ZnSO₄, 0.025 M sodium acetate pH 4.5). Incubation was at 37°C for 30 min. After chilling, 25 μ g of carrier calf thymus DNA was added followed by 0.5 ml of 2 N perchloric acid. The control system (1.5 ml) consisted of 30 μ g of liver DNA, 10 μ g enzyme in KZS to which 25 μ g carrier DNA and 0.5 ml of 2 N chilled perchloric acid were added without incubating. The acid-insoluble material was collected by centrifuging at 17,000 rpm for 20 min. The A_{260} of the supernate was measured. The amount of DNA rendered acid-soluble by nuclease *S*₁ was obtained by subtracting the A_{260} of the control supernate from that of the corresponding experimental sample. This value represented the amount of DNA digested by nuclease *S*₁ and was used to calculate the percentage of hydrolysed DNA. ●, Native DNA, ○, denatured DNA.

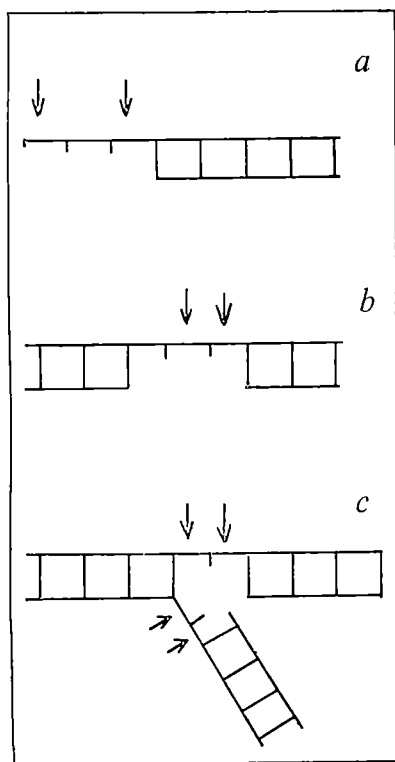


Fig 2 Postulated model of the regions of nuclease S_1 attack on the DNA of senescent mice: DNA with single-stranded region (a) terminally located, and (b) internally located, (c) cross-linked DNA with single-stranded regions in the junction region. Arrows indicate points of possible nuclease attack.

the formation of regional single-stranded stretches in the DNA of senescent animals represents a transitional stage in the process leading to the elimination of nucleotide sequences that are to be discarded¹. Figure 2 shows models of the possible structures of the regions in DNA attacked by nuclease S_1 . There may be many areas of nuclease S_1 attack on crosslinked DNA molecules of which we present a simpler model in Fig 2c. The metabolic significance of the existence of single-stranded regions in the DNA of older mice remains unknown. These regions may be the result of increased nuclease activity coinciding in time with a senescence-associated decline in repair enzyme activity in the cells. Loss in enzyme activity during senescence has been observed in other systems⁸. Thermodynamically, this has the advantage of making the energy previously reserved for repair functions available for hydrolytic processes. If the defective regions of the DNA occur in the genes for constitutive functions and are not repaired at the appropriate time, the biochemical lesions in the metabolic pathways of the particular cells are likely to result in the death of the organism, the harm can be particularly severe in the cells of non-replenishing tissues such as those of the muscular and nervous systems.

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Is mRNA transcribed from the strand complementary to it in a DNA duplex?

We have tested the assumption that the messenger RNA sequence is uniquely determined by the DNA sequence complementary to it. Because RNA is usually transcribed from a DNA duplex, there is the possibility that the DNA strand that is not complementary to the mRNA influences the RNA base selection. This possibility exists in models that postulate like-with-like base pairs¹⁻³, triple helix models of DNA-DNA-RNA⁴, triple base interactions such as those described in tRNA⁵, and models in which RNA polymerase may confer a special type of specificity (non-Watson-Crick) to RNA base-DNA duplex interactions, as well as in experiments in which separated are compared with non-separated strands⁶⁻⁷.

The logic of our experiment is as follows. If one makes DNA heteroduplexes in which one strand has the coding for the premature termination of a given protein, while the other has the coding for the complete protein, and then uses these heteroduplexes as templates for transcription-translation in a coupled⁸ cell-free protein synthesizing system, then one can ask, by fractionating the radioactive protein products on Ornstein-Davis⁹⁻¹⁰ acrylamide gels containing SDS, which product is made—the complete protein, a fragment or neither? If coding for RNA synthesis were solely a function of the nucleotide sequence of only one strand of a DNA duplex, then no matter

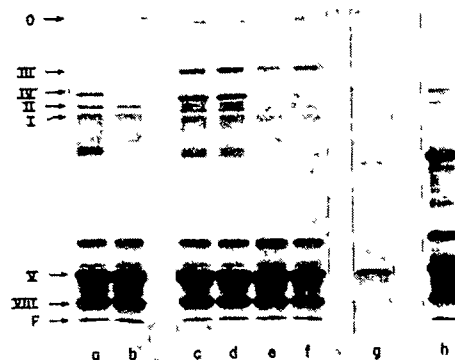


Fig 1 Autoradiograph of urea SDS-Tris-glycine acrylamide gels showing the *in vitro* protein products stimulated by various homo- and hetero-duplex DNAs. Gene products were identified as described before¹⁸ and are marked with Roman numerals. DNAs used as template for incorporations shown in (a), (b) and (h) were homoduplex (had not been subjected to cleavage, denaturation and renaturation). DNAs used were (a) WT homoduplex, (b) amber IV₁₂ and VII homoduplex, (c) amber IV₁₂ and VIII +, WT -, (d) amber IV₁₇ +, WT -, (e) WT +, amber IV₁₂ and VII -, (f) WT +, amber IV₁₇ -, (g) background (no added DNA: the bands reflect the synthesis of *E. coli* proteins from endogenous message), (h) amber IV₁₇ homoduplex, showing prominent fragment produced when this DNA is used as template. DNA was isolated as described before¹⁸. Heteroduplex molecules were constructed by cleaving RFI with endonuclease R *Hind*, isolating the cleavage products on sucrose gradients, denaturing the product in alkali, annealing to a 20-fold molar excess of the appropriate phage (+) strand DNA, and re-annealing at 65°C for 1.5 h, as described by Vovis *et al.*¹³⁻¹⁴. The circular reannealed molecules were purified by neutral sucrose gradient centrifugation and used as templates in the coupled transcription-translation system. All subsequent steps were as described previously¹⁸.

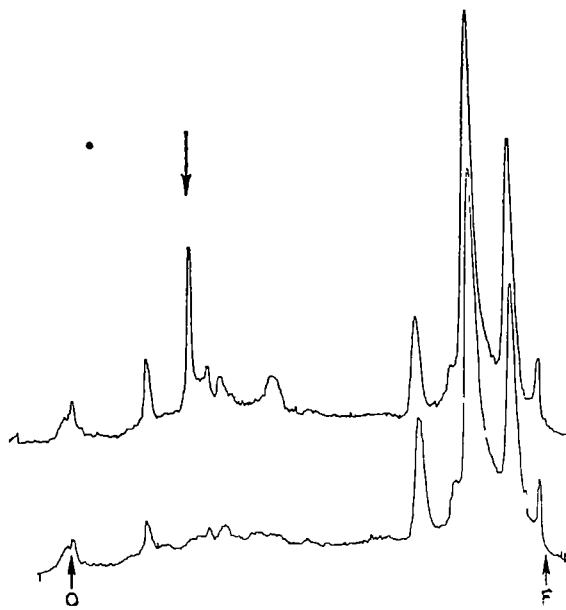


Fig. 2 Densitometer tracing of an autoradiograph of protein products programmed by heteroduplex DNA of composition +, wild type, -, amber IV (lower tracing), +, amber IV, -, wild type (upper tracing). Other details are as described in Fig. 1. The arrow marks the position of the gene IV product.

which or how many termination signals were on the other strand a complete protein would be made from at least one of the two possible heteroduplexes. Furthermore, if the results indicate that only one strand actively codes for RNA and the resultant protein, one can determine which strand is active.

To test these hypotheses, we constructed heteroduplex DNA molecules using coliphage ϕ 1 DNA. RNA transcribed from this DNA, both *in vivo* and *in vitro*, is complementary to only one of the DNA strands, the non-phage or (-) strand¹¹⁻¹². The heteroduplexes were constructed by annealing plus strands obtained from phage with minus strands obtained from intracellular double-stranded-replicative form DNA (RFI) as described by Vovis *et al.*¹³⁻¹⁴. Six types of heteroduplexes were constructed using two distinct gene IV amber mutants, these heteroduplexes included all permutations of amber and wild type genes in both plus and minus strands.

As Figs 1 and 2 show, complete proteins are synthesised by the coupled transcription-translation system whenever the minus strand of the heteroduplex is wild type with regard to gene IV. When the minus strand contains a gene IV amber mutation, the gene IV product is not made. Because of intrinsic sensitivity limitations of the assay system, we would not have been able to detect effects on protein synthesis comprising less than 10% of the wild type control.

These results thus support the hypothesis that the information for RNA transcription of structural genes is not strongly affected by a single base change in the duplex strand not complementary to the mRNA synthesised. We do not know what the effect of other sorts of changes would be.

All of the heteroduplex molecules were constructed by a method which includes the use of endonuclease R *Hind* to cleave ϕ 1 RFI. This endonuclease makes a single, unique double strand break in this DNA¹⁵, which is located within gene II¹⁶⁻¹⁷. Such cleaved DNA does not act as template for the synthesis of gene II protein *in vitro*¹⁸. It is interesting then, that some gene II product was synthesised in reactions primed by the circular heteroduplex DNA molecules which initially contained a single-stranded break within gene II. This suggests that transcription may not be affected by the presence of a nick in the template strand, although further experiments are needed to establish the point firmly.

This experiment was designed to test the transcription rules for structural genes coding for proteins, and showed that only the template strand seems to be significant DNA which is

heteroduplex in regions corresponding to promoters, operators or transcription termination regions might not function normally, since in these instances DNA-protein recognition is quite likely to involve both strands of a duplex, and there is no *a priori* reason to think that function is limited to one strand.

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Template activity of ϕ 1 RFI cleaved with endonucleases R *Hind*, R *Eco*P1 or R *Eco*B

ENDONUCLEASE R *Hind* makes a single, unique, double-stranded break in the double-stranded circular DNA (RFI) which can be isolated from bacteria infected with bacteriophage M13 (ref. 1), ϕ d (refs 2 and 3), or ϕ 1 (Horiuchi and Zinder, unpublished observations). We wish to report that ϕ 1 RFI cleaved by endo R *Hind* acts as a template for protein synthesis in a coupled *in vitro* transcription-translation system⁴, and that with the exception of the product of gene II of ϕ 1, the normal complement⁵ of *in vitro* phage-specific proteins is made. No prominent new polypeptide is observed (Fig. 1), this suggests that a substantial fragment of the gene II product is not coded for by RFI cleaved with endo R *Hind*. In addition, neither the size nor the relative yield of the product of the proximal gene^{5,6}, gene IV, is affected. This implies that the break is likely to be in a region extending from the C-terminus of gene IV to the N-terminal portion of gene II. A similar location has been assigned to this break with the use of entirely different methods by Seeburg and Schaller⁷.

Endo R *Hind* is one of a class of endonucleases which cleave DNA at specific sites and do not require a specific cofactor^{8,9}. The full length, linear, double-stranded DNA

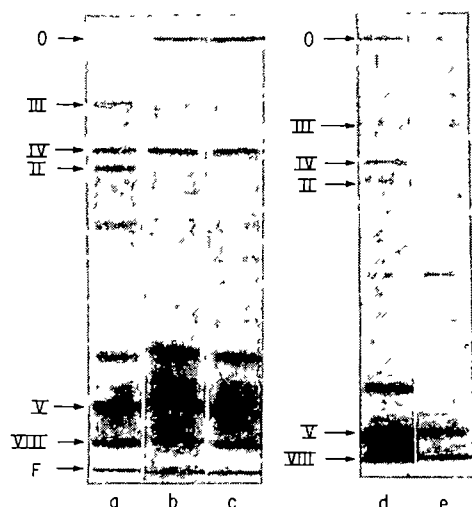


Fig. 1 Autoradiographs of urea-Tris-glycine-SDS acrylamide gels showing the protein products synthesised *in vitro* in a coupled system⁴ with the use of f1 RFI (a), f1 RFI cleaved with endonuclease R *Hind* (b), or with endonuclease R *Eco*PI (c), or with endonuclease R *Eco*B (d). A background incorporation (no added DNA) is shown in (e), and is to be compared with (d) only. Roman numerals identify gene products, as in ref. 5 (a), (b) and (c) are from the same gel slab, (d) and (e) from a different gel slab. In (d) the coat protein, product of gene VIII, has migrated close to or at the front, and is not readily distinguishable from it. RFI was prepared as before⁵, subjected to cleavage as detailed in the legend to Fig. 2, and used as a template as described before⁵. Electrophoresis, autoradiography and so on were as described before⁵. O marks the origin, F the front.

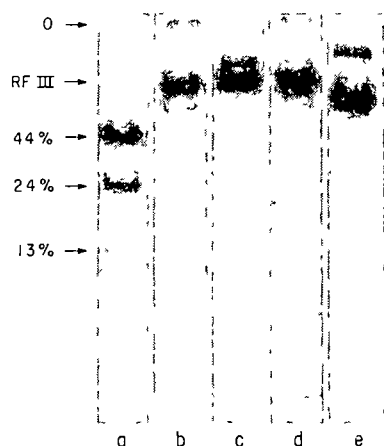


Fig. 2 Autoradiograph of a 14% agarose gel showing a, Three fragments generated from f1 RFI by endonuclease R *Hae*III, as DNA size markers. The size of each fragment is calculated from its relative content of ³²P, and is expressed as a percentage of the total f1 genome. Fragment sizes from Horiuchi, unpublished; b, Endo R *Hind* cleavage product of f1 RFI previously cleaved with endo R *Eco*PI (RFIII/PI); c, f1 RFI cleaved with endo R *Eco*PI only; d, f1 RFI cleaved with endo R *Hind* only; e, Untreated f1 RFI (the upper band is contaminating RFII). Cleavage reactions with *Haemophilus* endonucleases were carried out as follows: a 20 µl reaction contained 7 mM Tris-HCl, pH 7.4, and 7 mM MgCl₂, 0.2 µg ³²P-f1 RF and either 0.6 µg endo R *Hae*III or 1.3 µg endo R *Hind*. Incubations were at 37°C for 1 h. Reactions were stopped by adding 50 mM EDTA-0.25% SDS and the mixture was analysed by electrophoresis on 14% agarose gels¹². Cleavage reactions with endo R *Eco*B and R *Eco*PI were carried out as described before¹⁰ and the RFIII generated in these reactions was isolated and purified as described¹⁰. The RFIII was dialysed against 0.05 M Tris, 0.05 M NaCl, 0.001 M EDTA, pH 7.4, concentrated under a stream of N₂ gas, precipitated with 3 volumes of 95% ethanol, redissolved in the above buffer and used either as template for protein synthesis or as substrate in further restriction enzyme reactions.

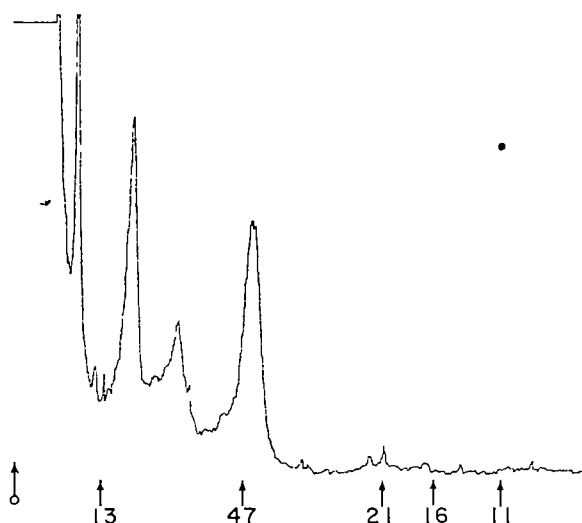


Fig. 3 Densitometer tracing of an autoradiograph of a 4% acrylamide gel slab on which the products generated from ³²P f1 RFI by the action of endo R *Eco*PI and R *Hind* had been subjected to electrophoresis. The origin is marked O, the direction of electrophoresis is from left to right. Larger fragments stay at or near the origin, smaller ones migrate into the gel. The arrows indicate the position at which fragments generated by endo R *Hae*III migrated in another slot of the same gel. The numbers under the arrows refer to the sizes of these marker fragments, expressed as a percentage of the f1 genome. All other conditions were the same as those in Fig. 2, and the material placed on the gel was the same as that shown in Fig. 2b. Gels were prepared and run as described by Danna *et al.*¹³

(RFIII) produced from f1 RFI by the action of endo R *Hind*, when denatured and subsequently reannealed, remains a linear, full length molecule¹. We have previously shown¹⁰ that in the presence of S-adenosyl methionine and ATP, endo R *Eco*B and R *Eco*PI also yield full length linear molecules as their primary product, but, in contrast to those produced by endo R *Hind* these molecules form circular structures upon denaturation and renaturation. This observation suggested that the sites of cleavage of these latter enzymes are not unique, even though only one double-strand break is introduced into any particular molecule¹⁰.

It was interesting, then, to use f1 RFIII produced by these enzymes as templates for the coupled system. As Fig. 1 shows, endo R *Eco*PI, like endo R *Hind* creates an RFIII which is no longer able to act as template for the synthesis of gene II product. DNA cleaved by endo R *Eco*B seems still to code for the normal complement of f1 *in vitro* proteins, but at much reduced efficiency. In view of the postulated heterogeneity of the cleavage sites for endo R *Eco*PI (ref. 10), the specific absence of gene II protein was rather surprising. Our dilemma was resolved, however, by examination of the cleavage products obtained when RFI is treated both with endo R *Eco*PI and R *Hind*. Figure 2 shows that stepwise treatment of f1 RFI with endo R *Eco*PI and then endo R *Hind* generates a primary product which is not distinguishable, in mobility on 14% agarose gels, from that produced by endo R *Hind* alone. Longer exposure of the autoradiographs shows that in addition, two minor components are found as products of the combined cleavage, one comprising some 70% and the other about 30% of the genome. When the products of the stepwise cleavage are analysed on 4% acrylamide gels, which resolve smaller fragments, four additional fragments are observed which range in size between 4 and 16% of the f1 genome (Fig. 3). We interpret these results to mean that most of the endo R *Eco*PI mediated breaks occur at positions which are within a distance of 4 to 16% from the site of the endo R *Hind* break. The other cleavage products of the combined action of the two enzymes, those ranging in size from 84 to 96% would not be readily

distinguishable from the full length RFIII in the agarose gels (Fig 2) From the protein synthesis data, we infer that most of the endo R *Eco*P1 breaks occur within gene II, which spans, at a minimum, some 20% of the f1 genome⁵ The site which gives rise to the 70 and 30% fragments is most probably not within gene II

Takanami¹¹ has demonstrated that *in vitro* transcription of fd RFI leads to at least four well defined transcripts Use of fd RFI cleaved by endo R *Hind* diminished the size of the two larger transcripts, but did not affect the size of the two smaller² These results of Takanami², which we have confirmed, together with the data presented here, suggest that only the larger of the RNAs participate in the synthesis of gene II protein

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Induction of dominant lethal mutation in male mice by ethyl alcohol

THERE have been many studies of the effects of ethyl alcohol on reproductive performance and of the genetic aspects of this relationship¹⁻¹⁰ But in spite of extensive investigations of the genetic determination of alcohol preference in experimental animals, there has been little research on other genetic aspects of alcohol consumption¹¹⁻¹³ It has been assumed for a long time that alcohol presents no genetic hazard Using the dominant lethal technique, we have now found that in mice ethyl alcohol can be mutagenic when given under certain conditions

We carried out three experiments to investigate the mutagenic role of ethyl alcohol in male mice In the first (done in the Department of Genetics, University of Cambridge, England) eight 10-week-old CBA/Fa Cam male mice were given 0.1 ml of 40% ethyl alcohol (1.24 g g⁻¹) by polyethylene gastric tube once a day for 3 consecutive days

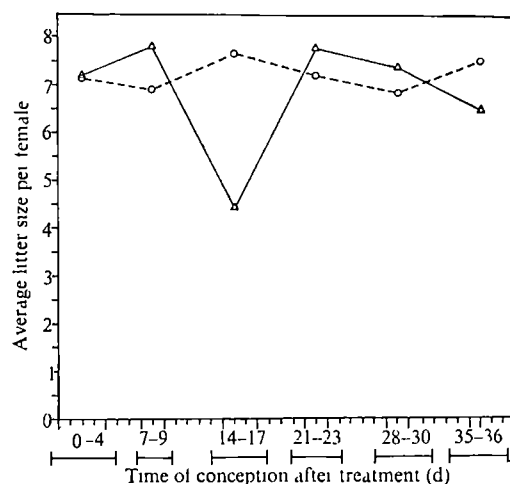


Fig 1 Effect of ethyl alcohol on litter size sired by treated males ○, control, △, treated

Each mouse was then caged with a virgin, untreated female of the same strain On the seventh day the males were transferred to cages with different virgins Five treated males were allowed to mate with new females at weekly intervals until the sixth week Pregnant females were allowed to produce their first litters, the size and sex ratios of which were recorded The young were examined for morphological abnormalities Several matings of the breeding colony were set up at the same time and in the same way but without alcohol treatment, to serve as a control

In our second experiment (carried out at the Worcester Foundation for Experimental Biology) mice of the inbred strain CBA/J (Jackson Laboratory) were tested for mutagenicity of ethyl alcohol using the dominant lethal test Mice were raised at the Worcester Foundation from the age of 7-10 weeks before use Two groups of male mice were given ethyl alcohol by gastric tube as before In one group, 13 males were treated with 0.1 ml of 40% alcohol daily for 3 consecutive days The second group of six males were each treated as above but with 0.1 ml of 60% (1.88 g g⁻¹) alcohol The eight control males were each given 0.1 ml of distilled water After the third treatment, each was mated to two CBA/J females and to further females after 3 d Each male was allowed to mate with new females seven more times at 4-d intervals The results of such a mating schedule would reflect the differential response of the different maturation stages of the germ cells

Successful matings were established by observing the formation of the vaginal plugs Pregnant females were killed and dissected 13-15 d after conception Corpora lutea and

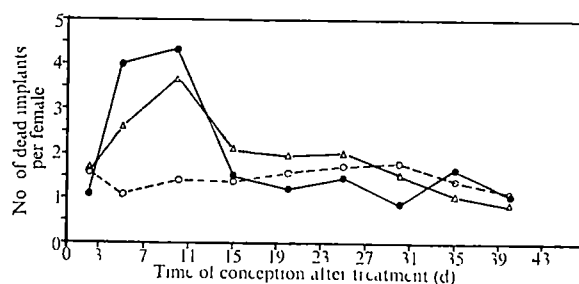


Fig 2 Dominant lethal induction in male mice with ethyl alcohol Effect on dead implants ○, control, △, treated with 40% alcohol, ●, treated with 60% alcohol

Table 1 Size of litters sired by male mice treated with 0.1 ml of 40% ethyl alcohol

Group of animals	Time of mating after treatment (d)	No of females mated	Frequency of fertile mating (%)	Litter size (mean \pm s.e.)	Dominant lethal* mutation index
Control	0-42	60	95	7.2 \pm 0.42	—
Treated with alcohol	0-4	10	100	7.8 \pm 0.36	-8.3
	(1st week)				
	7-9	10	100	7.5 \pm 0.50	-4.2
	(2nd week)				
	14-17	10	100	4.4 \pm 0.80†	38.3
	(3rd week)				
	21-23	10	90	7.7 \pm 0.33	-6.5
	(4th week)				
	28-30	10	100	7.3 \pm 0.92	-1.4
	(5th week)				
	35-36	10	100	6.4 \pm 0.48	11.1
	(6th week)				

*Dominant lethal mutation index

$$= \left[1 - \frac{\text{Litter size per experimental female}}{\text{Litter size per control female}} \right] \times 100$$

†t test for comparison between experimental and control values ($t=3.145$, $P<0.01$)

dead and live implants were scored for each pregnancy. Dead implants refer to early deaths. Late embryonic deaths were included with the live implants. The frequency of induced dominant lethal mutations was calculated according to Ehling *et al.*¹⁵

To assess the reproducibility of our results we repeated the second experiment, testing the effect of only one dose of alcohol, using CBA/J mice. Twelve males were treated with 40% alcohol. Twelve control mice received water instead of alcohol.

The size of full term litters obtained in the first experiment gives an estimate of the induction of dominant lethals as shown in Table 1. The number of live born approximates closely the number of live implants observed at mid-pregnancy except for late deaths. The latter has been found to be similar in mice treated with mutagenic agents and their control.¹⁶⁻¹⁸

The frequency of fertile matings in both control and treated groups seems not to be affected, particularly in the latter. Treatment of female mice of the same strain with the same dose of alcohol but for a longer period did not affect their fecundity (F.B., R.S.B. and M.E. Wallace, unpublished results). Prolonged treatments with larger doses were, however, reported to have some effect.¹¹

There is a close similarity between the average litter sizes of the control and the treated groups, with the exception of litters produced from matings that took place 14-17 d after treatment (Fig. 1). The difference in litter size of that group and the control is significantly different ($t=3.15$, $P<0.01$). The estimated dominant lethal mutation index is 38.3. The results suggest a considerable loss during prenatal life which could be, in large measure, the result of the induction of dominant lethals by alcohol. The results also suggest that different stages of germ cell maturation respond differently.

Although the matings were established at specific times, the entire period necessary to discern the precise pattern of differential response through spermatogenesis was not covered. A similar effect of alcohol on prenatal mortality has been reported with guinea pigs¹⁴, mice⁶ and rats¹².

A male that mated 10 d after cessation of treatment sired one young with abnormal bulgings on the head. The mouse was underweight for its age and died a few days after birth. All of its sibs were normal. The mother of the deformed mouse mated with the same treated father produced three more litters, none revealing similar or other types of abnormalities. It would be premature to conclude that this abnormality was due to alcohol treatment of the father.

Various abnormalities have been reported among progeny of animals treated with alcohol but in no case was there evidence that the alcohol caused any genetic changes.^{7,13,19}

Our second and third experiments were designed to obtain more specific information on the role of ethyl alcohol as a compound potentially able to induce dominant lethal mutation in the mouse. The two sets of data obtained from each experiment were analysed separately. The homogeneity of the data derived from the two experiments was

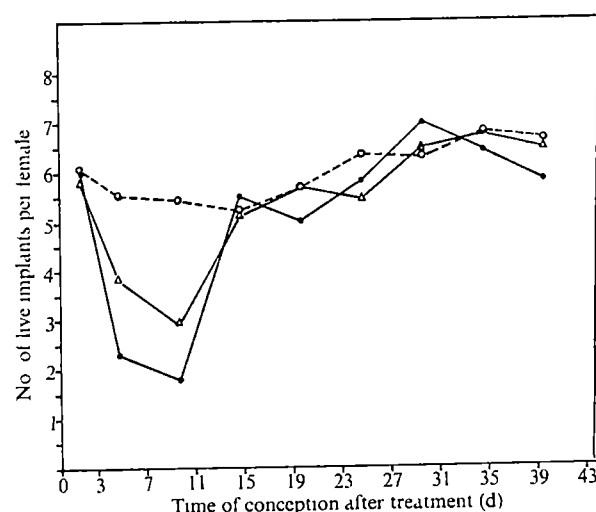


Fig. 3 Dominant lethal induction in male mice with ethyl alcohol. Effect on live implants. Symbols as in Fig. 2.

also tested and no statistical differences were found. This justified pooling the results of these two experiments presented in Table 2. The number of eggs shed, as represented by the number of corpora lutea at mid-term pregnancy, was not significantly different in the control and treated groups. Similarly, there was no significant difference in the number of total implants between control and treated groups.

The most striking observation is the significant (two to fourfold) increase in the number of dead implants in females mated 4-13 d after treatment of males with alcohol.

Table 2 Induction of dominant lethal mutation by ethyl alcohol in male mice

Treatment	Time of mating after treatment (d)	No of females with implants	Corpora lutea per female (mean \pm s.e.)	Total implants per female (mean \pm s.e.)	Dead implants per female (mean \pm s.e.)	Live implants per female (mean \pm s.e.)	Dominant* lethal mutation index
Control							
Water	1-43	165	9.0 \pm 0.2	7.5 \pm 0.3	1.5 \pm 0.2	6.0 \pm 0.3	
40% Ethanol	1-3	36	8.7 \pm 0.3	7.5 \pm 0.4	1.7 \pm 0.2	5.8 \pm 0.4	4.9
	4-8	33	9.5 \pm 0.2	6.5 \pm 0.6	2.6 \pm 0.2†	3.9 \pm 0.6†	30.6
	9-13	36	9.2 \pm 0.3	6.6 \pm 0.6	3.7 \pm 0.3†	2.9 \pm 0.6†	46.3
	14-18	38	9.1 \pm 0.3	7.2 \pm 0.5	2.1 \pm 0.3	5.1 \pm 0.6	1.9
	19-23	32	8.7 \pm 0.3	7.7 \pm 0.4	2.0 \pm 0.3	5.7 \pm 0.6	0.0
	24-28	26	9.4 \pm 0.2	7.4 \pm 0.6	2.0 \pm 0.3	5.4 \pm 0.4	15.0
	29-33	23	9.8 \pm 0.3	8.0 \pm 0.4	1.5 \pm 0.4	6.5 \pm 0.5	-3.2
	34-38	22	9.5 \pm 0.4	7.7 \pm 0.5	1.0 \pm 0.2	6.7 \pm 0.5	1.5
	39-43	24	9.5 \pm 0.4	7.3 \pm 0.7	0.8 \pm 0.2	6.5 \pm 0.5	3.0
60% Ethanol	1-3	13	9.0 \pm 0.4	7.1 \pm 0.7	1.1 \pm 0.4	6.0 \pm 0.7	7.7
	4-8	8	9.3 \pm 0.6	6.3 \pm 0.4	4.0 \pm 0.8*†	2.3 \pm 0.8*†	57.4
	9-13	11	8.9 \pm 0.4	6.1 \pm 0.7	4.3 \pm 0.4*†	1.8 \pm 0.6*†	67.3
	14-18	8	9.5 \pm 0.5	7.0 \pm 0.8	1.5 \pm 0.3	5.5 \pm 0.5	-14.6
	19-23	12	9.0 \pm 0.4	6.2 \pm 0.5	1.2 \pm 0.1	5.0 \pm 0.8	-2.0
	24-28	7	8.4 \pm 0.7	7.2 \pm 0.6	1.4 \pm 0.2	5.8 \pm 0.6	4.9
	29-33	8	8.7 \pm 0.7	7.8 \pm 0.6	0.8 \pm 0.1	7.0 \pm 0.8	-11.1
	34-38	11	8.6 \pm 0.3	8.0 \pm 0.7	1.6 \pm 0.2	6.4 \pm 0.7	4.5
	39-43	9	8.0 \pm 0.3	6.8 \pm 0.7	1.0 \pm 0.3	5.8 \pm 0.3	0.0

*Dominant lethal mutation index

$$= \left[1 - \frac{\text{Live implants of experimental group per female}}{\text{Live implants of control group per female}} \right] \times 100$$

†Statistically significant from the control values, $P < 0.01$

This considerable increase in post-implantation death is associated with a proportional decrease in the number of live embryos. These differences between the treated and control groups were statistically significant.

The dominant lethal mutation index was calculated using the formula given in Table 2. The values were based on comparison of the experimental groups of a specific mating interval and their concurrent control group. The estimated dominant lethal mutation index increased for matings between days 1 and 13 in the 40% group, reaching a peak during days 9 to 13. For groups treated with 60% alcohol, the increase in dominant lethal mutation index was rapid and abrupt with much higher frequencies at the mating intervals of 4-8 and 9-13 d. These frequencies (57.4 and 67.3) are higher than for groups treated with 40% alcohol (30.6 and 46.3) at the same mating intervals, which suggest in turn a positive dose response relationship. These results show that ethyl alcohol in the doses used induced dominant lethal mutations in several different spermatogenic stages, namely epididymal spermatozoa and late spermatids. The efficiency of induction is, however, more pronounced in the late spermatid stage.

Although the results derived from both experiments, the one based on litter size and the other on uterine contents, point to differences in the most sensitive stages of spermatogenesis both tests reveal the mutagenic nature of ethyl alcohol.

Dominant lethal mutations have been regarded as imposing no genetic hazards on man since they merely result in abortions. Whether ethyl alcohol could produce a wider range of genetic effects beside dominant lethals, needs further investigation. A pilot study on heavy drinking males indicates a significantly higher correlation between the incidence of birth defects and the drinking behaviour of the fathers (F.M.B., S. Beaton and R. Trudell, unpublished). Association between maternal alcoholism and aberrant morphogenesis in the offspring has also been described recently²⁴.

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Evidence for postmeiotic effect of *t* factors causing segregation distortion in mouse

THE *t* complex of the mouse consists of a large series of genetic factors often characterised by embryonic lethality of the homozygotes, reduced male fertility of viable homozygotes, suppression of recombination over a relatively long chromosomal segment and relatively frequent occurrence in natural mouse populations. Most *t* factors also possess a component which interacts with the *Brachyury* (*T*) gene and causes complete absence of the tail in *T/t* heterozygotes. One of the most fascinating but least explored characteristics of the *t* factors is their effect on genetic segregation. A male mouse carrying a

Although this segregation distortion has been known for almost half a century, it has not been satisfactorily explained. It has been suggested that the distortion may be due to abnormal meiotic divisions (meiotic drive¹). More specifically, it may be due to a process in which homologous chromosomes distribute themselves unequally during metaphase I of male meiosis with the result that the spermatocytes receive one chromosome of a given pair more frequently than the other. Here we present evidence that in a *t/+* male, the *t* and the *+* bearing chromosomes are distributed during metaphase I in a 1:1 ratio, implying that the actual distortion must occur after metaphase I, most likely postmeiotically.

The evidence for the meiotic effect of *t* factors was obtained by cytogenetical means, using a strain of mice recently produced in this laboratory and carrying Robertsonian translocation *T(16,17)7Bn*, abbreviated *T7* (ref. 2). The strain was derived by repeated backcrossing of hybrids between the house mouse (*Mus musculus*) and the tobacco mouse (*M. poschiavinus*) to the inbred strain C57BL/10, and selection for chromosome 17 carrying the *t* complex. During the selection, six of the seven metacentrics present in the tobacco mouse were eliminated and only the one composed in part of chromosome 17 was maintained. The *T7* translocation is easily identifiable both at mitosis (as a single or double metacentric among 39 or 38 telocentrics, depending on the zygosity of the carrier) and meiosis (as a quadrivalent at metaphase I and a metacentric chromosome at metaphase II).

The rationale for the present experiment was as follows. If the segregation distortion is caused by meiotic drive during metaphase I, at metaphase II a *T7+/+t* male should show a preponderance of meiotic figures lacking the metacentric marker, if, on the other hand, the distribution of homologous chromosomes at metaphase I is normal, a 1:1 ratio of figures with and without the metacentric can be expected at metaphase II.

The prerequisite of such an experiment is that the *T7* translocation itself does not greatly disturb the segregation of the homologues at meiosis. For this reason, males carrying the *T7* marker but not the *t* factor were also tested.

Table 1 Frequency of metaphase II spreads with one metacentric chromosome in *T7+/+t* males

Animal Number	1M*	OM†	Total	%1M‡	χ ²
199/919	9	12	21	42.9	0.42
199/920	32	37	69	46.4	0.32
199/921	11	17	28	39.3	1.28
199/922	28	36	64	43.8	1.0
199/923	27	32	59	45.8	0.42
199/924	21	27	48	43.8	0.76
199/925	19	30	49	38.8	2.46
200/1151	7	11	18	38.9	0.88
200/1334	29	41	70	41.4	2.06
Total	183	243	426	43.0	8.4

* No metaphase II spreads with one metacentric (*T7*)

† No metaphase II spreads without the metacentric

‡ % spreads with one metacentric

t factor in a heterozygous condition (*t/-*) often transmits the factor to almost 100% of its progeny, in defiance of the Mendelian principle requiring 1:1 segregation.

Table 2 Comparison of results with different *t* factors

Genotype	No of males tested	Frequency of metaphase II spreads with one metacentric chromosome in males of indicated genotype				Segregation of the metacentric chromosome to offspring of males tested for the presence of this chromosome in metaphase II				Segregation of <i>t</i> factor in the absence of the metacentric chromosome	
		1M/ <i>T</i> *	%1M†	χ ² ‡	χ ² §	1M/ <i>T</i>	% progeny with 1M	χ ² ¶	χ ² **	Ot/ <i>T</i> ††	%Ot‡‡
+/+/+	1	4/69§§	5.8	54.0	46.4						
<i>T7</i> +/+/+	1	57/122	46.7	0.52							
<i>T7</i> +/+ <i>T</i>	5	70/155	45.2	1.46	0.15	73/137	53.3	0.60			
<i>T7</i> +/+ <i>t</i> ^{w2}	1	21/47	44.7	0.54	0.08	1/18	5.6	14.2	0.47	15/137	10.5
<i>T7</i> +/+ <i>t</i> ^{w5}	5	85/116	42.3	4.8	1.57	2/67	3.0	59.2	3.03	4/44	9.1
<i>T7</i> +/+ <i>t</i> ⁶	9	183/426	43.0	8.4	2.38	10/177	5.6	139.2	65.92	25/72	34.7
<i>T7</i> +/+ <i>t</i> ¹²	4	78/170	45.9	1.16	0.04	2/92	2.2	84.2	23.88	49/205	23.9

* Number of metaphase II spreads with one metacentric (*T7*)/total number of scored spreads

† % spreads with one metacentric

‡ Observed frequencies of 1M spreads were compared to expected frequencies (that is 50%)

§ Observed frequencies of 1M spreads were compared to observed frequencies of 1M spreads in *T7*+/+/+ male

|| Number of progeny with one metacentric/total number of progeny. Progeny arose from matings of mice with the genotype indicated in the first column to +/+/+ or +/+/+*T* mice. Presence or absence of the metacentric chromosome (*T7*) was determined in mitotic metaphase spreads.

¶ Observed frequency of progeny with *T7* from the matings of *T7*+/+*T* or *T7*+/+*t* × +/+/+ or +/+/+*T* was compared to expected frequency (that is 50%)

** Observed frequency of progeny with *T7* from the mating of *T7*+/+*t* × +/+/+ or +/+/+*T* was compared to observed frequency of progeny from the mating +/+/+ × +/+/+ or +/+/+*T* (see column 12)

†† Number of progeny without *t*/total number of progeny. Progeny arose from matings of +/+/+*t* or +/+/+*t* to +/+/+ mice. Segregation of + chromosome was detected by H-2 typing.

‡‡ % progeny without *t* from matings in the absence of *T7*

§§ As B10.A lacks the metacentric chromosome, the four spreads scored as 1M must be reading errors. Two telocentric chromosomes which happen to be positioned in the spreads close to each other with their centromeres almost in contact and their arms pointing in opposite directions resemble and are confused with a metacentric chromosome. Such a reading error, however, is so small that it does not influence the conclusions drawn from the data.

In the experiment, $T7+/T7+$ females were mated to $+t/+T$ males and two types of progeny were produced $T7+/+t$ (normal tail) and $T7+/+T$ (short tail). Both the normal tail and the short males were further mated to $+/+/+$ or $+/+/+T$ females, the progeny of these matings were killed, mitotic chromosome preparations were prepared from bone marrow³ and the segregation of the $T7$ chromosome was determined. This progeny test provided the necessary control for demonstrating that the t factor distorted segregation whereas the $T7$ translocation did not. If this were the case, one would expect the progeny of the $T7+/+T \times +/+/+$ mating to segregate in a 1:1 ratio of mice with and without the metacentric, and the progeny of the $T7+/+t \times +/+/+$ mating to have preponderance of mice without the metacentric. After a sufficient number of progeny were obtained from the $T7+/+T$ and $T7+/+t$ males, the animals were killed, meiotic chromosome preparations were made from their testes⁴, and the metaphase II spreads evaluated for the presence or absence of the metacentric chromosome. Twenty to twenty-five slides were made from each male and only metaphase II spreads of 19 telocentrics and one metacentric, or 20 telocentrics were counted. Four different t factors were tested t^6 , t^{12} , t^{w2} , and t^{w5} .

Although there was a considerable male-to-male variation in the individual experiments (Table 1), the overall results with the different t factors were comparable (Table 2). In the meiotic test (Table 2), the observed frequency of 1M spreads (that is metaphase II spreads with one metacentric chromosome) in $T7+/+$ mice was not significantly different from the observed frequency of 1M spreads in $T7+/+t$ mice. When the observed frequency of 1M spreads in $T7+/+t$ mice, however, was compared to the expected frequency (50%), significant difference was found for some t factors (t^6 and t^{w5}). The departure from the expected value was most likely caused by the presence of the metacentric chromosome. (The frequency of 1M spreads in $T7+/+$ animals was lower than the expected 50% value, but for the number of animals used, the difference was not statistically significant.)

In the progeny test (Table 2), two t factors (t^{w2} and t^{w5}) were transmitted in almost exactly the same ratios in $T7+/+t \times +/+/+$ matings as in $+T/+t \times +/+/+$ or $+/+/+t \times +/+/+$ matings. The other two factors tested (t^6 and t^{12}), on the other hand, were transmitted in significantly lower ratios in $T7+/+t \times +/+/+$ matings than in $+T/+t \times +/+/+$ or $+/+/+t \times +/+/+$ matings. This latter observation suggests that $T7$ interacts with t factors postmeiotically, resulting in an increased transmission ratio of t . This interaction, however, can be detected only with t factors that, in the absence of $T7$, have low (t^6) or moderate (t^{12}) transmission ratios. The nature of the interaction is not known.

We conclude that two types of segregation distortion operate in the experiments we describe, one a very minor type occurring during the first meiotic division and another which has a very strong effect postmeiotically. The meiotic distortion can most likely be attributed to the presence of the $T7$ translocation, whereas the strong distortion is caused by the t factors and it may be enhanced by the $T7$ chromosome. Thus, the distortion resulting from t factors does not occur in metaphase I as a result of a structurally abnormal chromosome but rather postmeiotically. This conclusion is in agreement with the data of the effect of late mating^{5,7}, which shows a normalisation of the transmission ratios as a result of the shorter period of time between insemination and fertilisation. Further evidence for the postmeiotic effect of t factors is provided by recent work⁸ in which epididymal sperm were incubated *in vitro* and the number of surviving sperm were estimated at intervals by a cytotoxic test using antisera directed against a specific t factor. They demonstrated that the number of t bearing sperm increases with time as the number of $+$ sperm decreases.

The data we present, however, do not rule out the possibility of a biochemical activity related to the t factors that may occur before or early in meiosis but have a postmeiotic effect as

has been suggested by Erickson⁷.

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Factor which affects the mode of genetic recombination in *E. coli*

THE normal recombination process in *Escherichia coli* requires a pathway involving a functional *recB* and *recC* gene product (henceforth designated the RecBC pathway)¹. The *recB* and *recC* genes map as closely linked cistrons near *thy* on the *E. coli* chromosome map (54 min)^{2,3}. ATP-dependent DNase (*recBC* DNase) has been identified as the product encoded by these genes⁴⁻⁶. Another gene essential for recombination proficiency, *recA*⁷, is located between *cysC* and *pheA* (51 min)⁸. Genetic transformation studies⁹⁻¹² showed that the most efficiently transformable *E. coli* strain (genotype *recB⁻ recC⁻ sbcB⁻*) lacks the DNase but retains recombination proficiency because of the *sbcB⁻* allele which acts as an indirect suppressor of the *recB⁻ recC⁻* mutations¹³. This strain uses a recombination pathway (designated as the RecF pathway)¹ which does not involve the ATP-dependent DNase but consists of products of several recombination genes¹³. Restoration of the ATP-dependent DNase to this strain by introduction of the *recB⁺ recC⁺* alleles reduced the transformation frequency by a factor of approximately five⁹. *In vitro* studies demonstrated that this DNase degrades linear DNA molecules extensively, while it has no effect on circular DNA⁴. The adverse effect of the DNase on transformation may therefore be due to destruction of linear donor DNA molecules before they reach the recombination machinery. These results raise the possibility that in normal cells the DNase affects the mode of recombination by selecting or destroying DNA molecules of a particular structure.

The transfer of genetic material can result in the formation of two possible types of recombinant clones: (a) substitution type resulting from replacement of recipient genetic material by a homologous segment of donor material, and (b) addition type

resulting from addition of the donor genetic material to the recipient chromosome. We describe here transduction and transformation experiments designed to examine the effect of the *recBC*-specified DNase on the two possible types of recombination. Similar experiments using λ *gal bio* were reported recently by Wackernagel and Radding¹⁴.

The genetic backgrounds of the *E. coli* K12 strains used in our study have been described¹⁰. M0639 is a tryptophan-requiring derivative (*trpB9579*)¹⁵ of JC7623 (*recB21 recC22 sbcB15*)¹⁶. Strains M0649 (*recB⁺ recC⁺ sbcB15*) and M0650 (*recB⁺ recC⁺ sbcB⁺*) were constructed from strain M0639 by conjugation with Hfr KL16.

We studied specialised transduction and transformation with ϕ 80pt and λ pt phages and their DNA¹⁷⁻²⁰. The ϕ 80pt and λ pt we used both lack the *int* and *red* genes which are replaced by part or all of the *trp* operon of *E. coli*. Two classes of Trp⁺ recombinants are generally observed in a single transduction (or transformation) experiment. One class of Trp⁺ recombinants consists of those lysogenised by donor phage by a mechanism similar to that of specialised transduction. Oka *et al.*²⁰ demonstrated that the entire ϕ 80pt genome integrates exclusively at *trp* operon of *E. coli* undergoing addition type recombination by the mechanism proposed by Campbell²¹. The other Trp⁺ class

between 10% and 20% (13.5% for ϕ 80pt, 17.0% for λ pt) of Trp⁺ recombinants were ϕ 80pt or λ pt lysogens, suggesting that these are the products of addition type of recombination. The rest (86.5% for ϕ 80pt, 83.0% for λ pt) were not lysogenic and therefore the products of substitution type of recombination. Second, in strain M0639 (recombination pathway RecF, no *recBC*-encoded DNase) the proportion of Trp⁺ recombinants which are ϕ 80pt or λ pt lysogens is drastically reduced to less than 1% of the total Trp⁺ recombinants so that almost all Trp⁺ recombinants result from substitution type of recombination. Third, in strain (M0649) (*recBC*-specified DNase present plus a functional RecF pathway) the proportion of the Trp⁺ recombinants harbouring ϕ 80pt or λ pt was the same level as that of M0650.

These results demonstrate that the presence of *recBC*-encoded DNase in the cells affects the fate of donor DNA and changes the proportion of two types of recombinants. Two possibilities can be considered to explain these results. First, the recombination process of the RecF pathway (*recB⁻ recC⁻ sbcB⁻*) results primarily in the substitution type of recombination, whereas the RecBC pathway (*recB⁺ recC⁺ sbcB⁺*) catalyses substantial amounts of both types (substitution and addition) of recombination. Second, the increase in the proportion of the

Table 1 Transduction of the *trpB* gene by ϕ 80pt and λ pt

Donor	Recipient strain	Genotype	Frequency of Trp ⁺ transductants ($\times 10^{-3}$)	Fraction of Trp ⁺ transductants harbouring the donor phage
ϕ 80pt	M0639	<i>recB⁻ recC⁻ sbcB⁻</i>	1.50	1/149 (0.7%)
	M0649	<i>recB⁺ recC⁺ sbcB⁻</i>	0.37	15/150 (10.0%)
	M0650	<i>recB⁺ recC⁺ sbcB⁺</i>	0.31	21/155 (13.5%)
λ pt60-3	M0639	<i>recB⁻ recC⁻ sbcB⁻</i>	5.40	1/155 (0.6%)
	M0649	<i>recB⁺ recC⁺ sbcB⁻</i>	1.80	17/155 (11.0%)
	M0650	<i>recB⁺ recC⁺ sbcB⁺</i>	0.56	27/155 (17.0%)

ϕ 80pt transduction was performed according to Oka *et al.*²⁰. λ pt transduction was performed as follows: overnight Luria broth cultures of the recipients were suspended in the same volume of 10 mM MgSO₄ and aerated for 15 min. The λ pt60-3 was added at a multiplicity of infection of 0.1 and the incubation was continued at 37°C for 20 min. The transduction mixtures for both ϕ 80pt and λ pt60-3 were appropriately diluted, plated on selection plates for Trp⁺ recombinants and incubated at 37°C for 48 h. The Trp⁺ transductants were purified by two single-colony isolations on Difco Bacto penassay agar plates before testing for phage-specific characteristics. More than 99% of the original Trp⁺ transductants remained Trp⁺ after the purification steps. The presence of the phage genome in the Trp⁺ recombinants was demonstrated by both the ability to produce the phages and immunity to clear-plaque mutants of the phages. For testing phage production the purified colonies were spotted on Penassay plates, irradiated with ultraviolet light and replicated on a plate having an overlay seeded with the indicator strain MRP1. A clear zone of lysis proximal to the spot was taken to indicate phage production. The test for immunity was performed by cross-streaking the Trp⁺ recombinants against ϕ 80c or λ c126. All the Trp⁺ recombinants immune to the clear plaque phages were induced by ultraviolet irradiation to produce phage whereas all sensitive recombinants produced no phage.

represents the substitution type recombinants in which only the portion of donor DNA bearing the *trp⁺* genes is introduced into the recipient genome. Such recombinants should not acquire ϕ 80- or λ -specific characteristics. Therefore testing Trp⁺ recombinants for the absence or presence of the phage-specific characteristics (phage immunity and inducibility) is a convenient method for determining the nature of the recombination process undergone.

We have tested for these characteristics in the Trp⁺ recombinants obtained from three isogenic Rec⁺ (*trpB⁻*) recipient strains. One recipient (M0650, *trpB⁻ recB⁺ recC⁺ sbcB⁺*) possesses recombination pathway RecBC and is dependent on the intact *recBC* gene-encoded DNase. The second recipient (M0639, *trpB⁻ recB⁻ recC⁻ sbcB⁻*) possesses only the RecF recombination pathway which functions without an intact *recBC*-DNase. The third strain (M0649, *trpB⁻ recB⁺ recC⁺ sbcB⁻*) possesses the two recombination pathways¹³. Table 1 presents the results of transduction experiments involving these strains with ϕ 80pt (*trpA⁺B⁺C⁺*) and λ pt60-3 (*trpA⁺B⁺C⁺D⁺E⁺*) and can be summarised as follows. First, in the wild type (M0650) where the DNase (encoded by *recBC*) is functioning,

addition type recombinants observed in the recipient with the *recBC*-encoded DNase (*recB⁺ recC⁺ sbcB⁺*, *recB⁺ recC⁺ sbcB⁻*) may be the result of decreased availability of a particular type of donor DNA molecule due to its degradation by the DNase. (For example, DNA with either total or partial linear structure, which is sensitive to degradation by the DNase⁴, may be the substrate for the substitution type of recombination. Circular DNA which would survive may be the substrate for the addition type of recombination.) We believe the second possibility is the case for the following reasons. First, the reduction of transduction frequency (3–10-fold) was always observed in the recipient cells with the *recBC*-encoded DNase (Table 1). Second, the frequency of spontaneous segregation of Trp⁻ cells from Trp⁺ lysogens due to the loss of the ϕ 80 and λ were equal (1.5% to 2%) in three recipient cells regardless of the recombination pathway(s) they possessed.

A similar experiment with a specialised transformation system was performed with intact ϕ 80pt (*trpA⁺B⁺C⁺*) DNA as donor and with the same recipients as used for the transduction experiment (except for strain M0650 which is non-transformable). Among the total Trp⁺ transformants the proportion which was

Table 2 Transformation with $\phi 80pt$ DNA

Recipient	Genotype	Frequency of Trp ⁺ transformants ($\times 10^{-6}$)	Fraction of Trp ⁺ transformants harbouring $\phi 80pt$
M0639	<i>recB⁻ recC⁻ sbcB⁻</i>	7.1	8/150 (5.3%)
M0649	<i>recB⁺ recC⁺ sbcB⁻</i>	1.4	104/144 (72.3%)

Specialised transformation was performed as described by Oishi and Cosloy²² using intact $\phi 80pt$ (*trpA⁺B⁺C⁺*) DNA as donor at a concentration of $10 \mu g \text{ ml}^{-1}$. Tests for phage-specific characteristics of the Trp⁺ transformants are described in the legend of Table 1.

the result of lysogenisation by the $\phi 80pt$ (*trpA⁺B⁺C⁺*) genome was distinctly higher with recipient strain M0649 than with strain M0639 (Table 2). This result is essentially the same as we obtained with specialised transduction. With both recipients a much larger proportion of $\phi 80pt$ -bearing Trp⁺ recombinants was obtained after transformation than after transduction, apparently due to a reduced frequency of the substitution type of recombination in transformation. This suggests that DNA molecules which enter the cell as a result of injection by phage tend to interact with the cell's constituents differently from DNA molecules which enter from a pool of naked transforming DNA, perhaps because of differences in structure or in the mode of entry. This interpretation is supported by the report²⁴ that for certain phage the presence of *recBC* DNase in recipient cells reduces the efficiency of transfection, which involves introduction of intact purified phage DNA, while it has little effect on plaque-forming efficiency by infection with intact phage particles.

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Host range of murine xenotropic virus: replication in avian cells

AN endogenous C-type virus with an unusual tropism has been isolated from New Zealand Black (NZB) and other mouse strains. Although it has the murine leukaemia virus (MLV) *gs-1* antigen and reverse transcriptase, it cannot be propagated in mouse cells. It is only infectious for cells foreign to the host species and has been termed xenotropic¹⁻³. In the course of host range studies, we have discovered that, in contrast to other mammalian C-type viruses, this murine xenotropic virus can cross class barriers and both infect and replicate efficiently in avian cells. In these studies, the NZB pseudotype murine sarcoma virus (MSV) (Table 1) was used as it provides a good marker (focus formation) for NZB virus infection and replication.

As illustrated in Table 1, the NZB pseudotype virus gave efficient focus formation in a variety of mammalian cells including human, rat, guinea pig, rabbit, cat, cow, American bear, lion and African water mongoose. Infection occurred also in deer, racoon, gazelle, and Rhesus monkey. In the latter two cell lines only virus replication but not focus formation was detected. Infected animal cell lines which did not demonstrate focus formation nor NZB pseudotype virus production were cocultivated with human cells and passed three times. Supernatants were then assayed again for xenotropic murine sarcoma virus. These human cell cultures were also cocultivated with NRK-Harvey cells to determine if any NZB C-type virus were present. In all cases, the absence of progeny virus production in the original cell culture was not reversed by these added measures.

The xenotropic pseudotype sarcoma virus showed neither focus formation nor replication in C/O chicken cells including those established from *chf*-negative embryos. On the other hand, foci and progeny sarcoma virus were detected in duck, pheasant and quail cells. The foci formed in duck cells differed morphologically from those produced by Rous sarcoma virus (RSV). The MSV focus was composed of elongated, piled up cells rather than the round cells principally seen in RSV foci (Fig. 1a and b). The progeny sarcoma virus maintained the host cell tropism of the parental NZB virus as well as the NZB type specific coat as determined by neutralisation testing using antiserum prepared against the NZB C-type virus^{2,3}. Duck cell cultures yielded the highest titres of progeny virus.

As replication of the NZB pseudotype sarcoma virus requires the presence of a non-focus forming 'helper' C-type virus^{4,6,7},

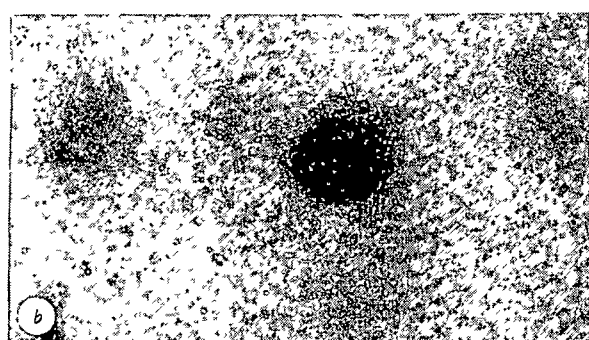
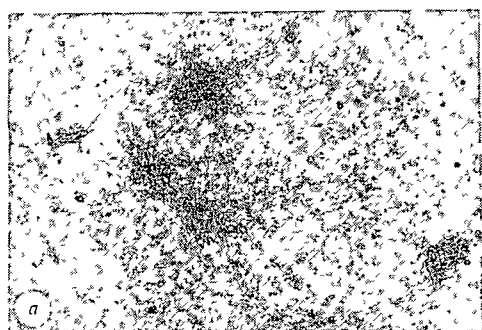


Fig 1 Focus of cell alteration induced in duck embryo cells, *a*, by the NZB pseudotype murine sarcoma virus (Note the elongated piled up fibroblast-like cells) ($\times 24$), *b*, by the Prague strain of Rous sarcoma virus (note the accumulation of primarily round cells) ($\times 24$)

xenotropic C-type virus must also be produced by these cells. Duck cells were infected directly with the C-type virus isolated from NZB cells and these cultures also yielded progeny NZB xenotropic virus. Duck cell cultures producing xenotropic MSV and/or MLV were superinfected by RSV (type C), and continuous cultures producing both types of viruses have been derived.

Xenotropic viruses isolated from NIH Swiss, C57L and C57BL mice share the same type specific coat as the NZB virus (refs 2 and 3 and P. Arnstein, J. A. L., L. S. Oshiro, P. Price, and E. Lennette, unpublished) and are also able to replicate in duck cells; these data indicate this ability to cross class distinctions is a general characteristic of the murine xenotropic virus(es).

The Harvey strain of murine sarcoma virus as well as Rauscher and AKR pseudotype sarcoma viruses were also tested for their ability to infect duck cells. No foci were detected and supernatants from the cultures did not yield any focus-forming virus nor any MLV as determined by the XC plaque assay⁸. The Kirsten strain of MSV (K1-MSV) has been reported to have a wide host range including human cells⁹⁻¹², and we have been able to transform duck cells with an isolate obtained from a continuous line of rat cells chronically infected with K1-MSV. Infection of human cells, however, has not been possible with all preparations of K1-MSV (J. A. L., unpublished, and S. Panem and W. H. Kirsten, unpublished). K1-MSV in some laboratories seems to lose its tropism for mouse cells after passage through heterologous hosts (ref 11 and 12 and V. Klement, personal communication). As suggested previously², it seems most likely, that those preparations of Kirsten MSV contain significant amounts of xenotropic virus and its pseudotype sarcoma virus and these viruses are responsible for the wide host range observed. Neutralisation of the progeny virus from these cultures with antiserum against xenotropic virus is needed to evaluate this possibility. Recently we learned that the isolate we received from Klement has been neutralised by antiserum to xenotropic virus and not antiserum to the parental Kirsten virus (V. Klement, personal communication). This result

suggests that any MLV with a wide host range is most likely from the xenotropic MLV subgroup.

The variability in extent of focus formation and degree of progeny production by the xenotropic pseudotype sarcoma virus indicates that cells differ in their sensitivity for infection by the virus (input response) and their ability to propagate the virus after infection (output response) (Table 1). For example, although the same number of foci can be induced by xenotropic pseudotype sarcoma virus in human and rat cells with approximately the same multiplicity of infection, human cell cultures yield ten times more progeny virus (Table 2) than the rat cell cultures. As MSV replication depends on the helper C-type

Table 1 Host range of xenotropic NZB-MLV

	FF titre*	Progeny production†
Mammalian		
Human foreskin	40	30
Rat-NRK kidney	33	20
Guinea pig embryo	33	30
Rabbit kidney	23	30
Cat embryo	+	20
Bovine embryo	+	30
Black footed mongoose	29	20
African water mongoose	20	16
Horse dermis	23	07
American bear lung	20	03
Lion kidney	10	NT
Black footed deer kidney	03	03
Raccoon uterus	02	—
Gazelle lung	—	09
Lesser anteater	—	05
Rhesus monkey heart (embryo)	—	06
Rhesus monkey kidney	—	—
African green monkey kidney	—	—
NIH Swiss mouse embryo	—	—
BALB/c embryo	—	—
Wild mouse kidney (San Francisco)	—	—
Chinese hamster embryo	—	—
Syrian hamster embryo	—	—
Peccary kidney	—	—
Bat lung	—	—
Avian		
Duck embryo (Pekin)	30	10
Ring-necked pheasant embryo	30	03
Quail	+	03
Chicken embryo (C/O)	—	—

Fertilised duck (Pekin) eggs and lymphomatous-free white Longhorn chicken eggs (C/O) were obtained from Kimberlin Farms (Freemont, California) and used at 10–12 d of age for preparation of primary embryo cultures. Other avian cells were provided by Dr Don Fugita (San Francisco). Cells from the mammalian species were provided by Dr W. Nelson-Rees (Naval Biomedical Laboratories, Oakland). All cultures were maintained and passed according to standard procedures¹⁻⁴.

*The stock of NZB pseudotype sarcoma virus used was made by cocultivating non-virus producing MSV-transformed rat cells, the NRK-Harvey cell line, with NZB embryo cells²⁻³. This virus preparation was adsorbed undiluted for 30 min (37°C) on to the cell monolayers pretreated with diethylaminoethyl dextran⁵. Cell cultures were read for focus formation (FF) on day 7 and 9. The sensitivity of the cell line is expressed by the titre of xenotropic pseudotype virus (\log_{10}) obtained: +, Distinct foci could not be detected; —, no foci detected.

†Nine-day supernatants from cell cultures receiving 10^4 focus-forming units of pseudotype virus were assayed for progeny virus on human foreskin cells by standard techniques¹⁻⁴. The titre is expressed as \log_{10} . NT, Not tested; —, no progeny virus detected.

virus, these observations suggest that successful infection by xenotropic viruses involves at least two mechanisms, first, at the cell surface before penetration (for example, the receptor level), and second, intracellular after virus penetration. The former depends on the viral envelope coat, the latter on the viral genome. An intracellular block has been proposed to explain the reduced ability of murine N and B tropic viruses to propagate in certain mouse cells¹³. A similar type of block probably limits the replication of xenotropic virus in rat cells,

and other animal cells in which only focus formation is efficient (Table 1) Preliminary results in our laboratory suggest a block at the receptor level prevents xenotropic virus infection of hamster cells, and a block in both mechanisms is responsible for the resistance of mouse cells to the murine xenotropic virus (J A L, unpublished)

Table 2 Comparison of progeny yield after infection by NZB pseudotype sarcoma virus

Cells*	Foci†	Progeny yield‡
NRK	150	8
Human foreskin	160	81

*Cells were inoculated at approximately the same multiplicity of infection

†Numbers represent the average number of foci in duplicate Petri dishes

‡Seven day supernatants from the cultures were assayed on human foreskin cells The average number of foci in duplicate plates is given

The growth of murine xenotropic virus in duck cells represents the first example of a C-type virus from one animal class replicating efficiently in cells from another animal class Svoboda and Klement reported tumours induced by RSV from which trace amounts of infectious RSV (ref 14) could be recovered Recently, the ability of the Schmidt-Ruppin strain of RSV to infect and replicate in kangaroo-rat cells¹⁵ has been described, but the efficiency of infection and replication by this RSV was much less than that of murine xenotropic virus in avian cells Moreover, after long term culture, the transformed marsupial lines tended to lose their capacity to produce virus¹⁵ On the other hand, duck cells, transformed by the NZB pseudotype virus have increased their virus production with passage and have yielded 10^5 – 10^6 focus-forming particles per ml as measured in either human, rat or duck cells

The xenotropic MSV can be used as a marker for molecular studies of cells producing both avian and murine C-type viruses Moreover, the sensitivity of certain avian cells to these murine C-type viruses provides another *in vivo* approach for studying xenotropic viruses and their possible role in normal development, evolution, autoimmunity and/or malignancy

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Delayed tumour appearance and absence of regression in nude mice infected with murine sarcoma virus

THE murine sarcoma virus (MSV) rapidly induces local tumours in mice^{1,2} The subsequent regression of such tumours in adult mice has been shown to be immunological in nature^{1,2} Young animals^{1,2} as well as animals immunosuppressed by X irradiation³, cortisol⁴, cyclophosphamide⁵, antilymphocyte serum⁶ or thymectomy^{2,7} have increased frequency of progressively growing tumours The immunological nature of these phenomena was also demonstrated by the transfer of resistance to tumour growth with cells³ or serum^{2,5,8} from animals that had regressing tumours The *in vitro* testing of lymphoid cells from mice with regressing tumours has indicated that both thymus-dependent (T)⁹ and non-T lymphocytes¹⁰ are generated as effector cells in this system

In the work reported here, the capacity to induce tumours and to produce spontaneous regressions of the developing tumours after MSV infection in adult *nu/nu* (nude-athymic) and *nu/+* mice (normal heterozygotes) was studied Nude mice have an abnormal development of the thymus and thymus-dependent systems and show a profound deficit of immune functions, especially of the thymus-dependent type¹¹

Nude mice partially inbred in a CBA/H background (5th to 6th backcross generation) and in BALB/c background (8th backcross, 2nd intercross generation) were used, as well as CBA/H or BALB/c mice (For details of origin of these strains and animal care see ref 12) All animals were free of lactic dehydrogenase (LDH)-elevating virus and had normal LDH serum values¹³, and all were injected intramuscularly at 50 d of age with 0.1 ml of Moloney strain MSV(M-MSV) In some experiments the *nu/nu* mice were treated with a thymus graft implanted intraperitoneally at 30 d of age (derived from 16–17-d-old CBA/H embryos or from BALB/c newborn donors) or with a thymus graft from CBA/HT6T6 origin enclosed in a diffusion chamber prepared with filters of a mean pore size of 0.10 μ m as described before¹⁴ Some experimental *nu/nu* mice were grafted at 30 d old with allogeneic C57BL/6 skin and these animals retained the grafted skin throughout the experiment All animals were observed for 50 d after M-MSV injection

Table 1 shows the results of such experiments M-MSV induced 100% local tumours in every experimental group, however, differences in the mean latent periods for local tumour development were observed While in the *nu/+*, CBA/H or BALB/c controls (experimental groups 1, 6, 7 and 12) the mean latent period ranged from 5.5 to 7.0 d, the latent periods for tumour development in the *nu/nu* groups (groups 2 and 8) were 19.1 and 16.8 respectively, which are significantly prolonged compared with any of the control groups The same prolonged latent periods for tumour development were observed in the two groups of *nu/nu* mice grafted with allogeneic skin to monitor immune incompetence (groups 3 and 9) Conversely, the *nu/nu* mice grafted with a free thymus graft (groups 4 and 10) behaved as the normal *nu/+* and had short latent periods for tumour development (7.1 and 6.9 d respectively) On the other hand, thymuses enclosed in a diffusion chamber were incapable of shortening the long latent period for tumour development in the *nu/nu* (group 5) A small additional group (group 11) of *nu/nu*-BALB mice injected intraperitoneally at 30 d of age with 100×10^6 cells from 60-d-old *nu/+* BALB thymuses showed

only a slight prolongation of the latent period for tumour appearance when compared with controls

Within the groups of *nu/+* mice, immune competence of some animals (16 in group 1 and 7 in group 7) was tested with grafts of C57BL/6 allogeneic skin at 30 d of age. Skin graft rejection within 12 d of grafting was observed in every instance (9.9 and 9.2 days respectively) and since such groups did not differ from the non-grafted animals in behaviour towards M-MSV infection, they were not considered as separate groups in Table 1.

Table 1 also shows the total incapacity of *nu/nu* mice to produce regressions of the developing tumours (groups 2, 3, 8 and 9). Conversely, the immunologically normal controls (groups 1, 6, 7 and 12) produced regressions in almost every instance within 10–12 d after tumour appearance. The thymus

suggesting that the target cell for the humoral activity of the thymus in the peripheral lymphoid tissues is a post-thymic cell which is absent in the *nu/nu* mice.¹⁴

The delay in tumour appearance in *nu/nu* may be explained by the inability of these mice to develop the early atypical granuloma which is a common feature of the M-MSV-induced tumours in normal mice.²³ Analysis of the cellular composition of the tumours appearing in an additional group of 5 *nu/nu* and 4 *nu/+* (on BALB/c background) supported this interpretation. The cellular composition, studied 7 d after tumour appearance (cells dispersed by 30 min incubation in 0.25% pronase) was 25–30% tumour cells in the *nu/+* versus 43–66% in the *nu/nu*, the remaining cells being lymphocytes, polymorphs and macrophages. The proportion of macrophages within the tumours (determined by ability to ingest latex particles) was

Table 1 Tumour incidence and regression in *nu/nu*, *nu/+*, CBA/H and BALB/c mice after injection of M-MSV

Experimental groups	No. with tumours per total injected	Mean latent period for tumour appearance (d ± s.d.)	No. of tumours regressing	Mean regression time (d ± s.d.)
(1) <i>nu/+</i> (CBA)	39/39	6.5 ± 1.9	39 (100%)	10.8 ± 2.1
(2) <i>nu/nu</i> (CBA)	21/21	19.1 ± 3.7	0	—
(3) <i>nu/nu</i> (CBA-Sk)	10/10	19.6 ± 2.5	0	—
(4) <i>nu/nu</i> (CBA-TG)	12/12	7.1 ± 1.4	11 (92%)	10.6 ± 1.6
(5) <i>nu/nu</i> (CBA-TDC)	10/10	16.9 ± 2.7	0	—
(6) CBA/H	20/20	7.0 ± 1.6	19 (95%)	12.1 ± 1.9
(7) <i>nu/+</i> (BALB)	16/16	5.5 ± 1.2	14 (87%)	10.2 ± 1.8
(8) <i>nu/nu</i> (BALB)	12/12	16.8 ± 2.4	0	—
(9) <i>nu/nu</i> (BALB/Sk)	7/7	17.2 ± 2.1	0	—
(10) <i>nu/nu</i> (BALB-TG)	12/12	6.9 ± 1.5	10 (83%)	10.6 ± 1.5
(11) <i>nu/nu</i> (BALB-Thy)	6/6	10.0 ± 2.1	2 (33%)	13.5 ± (12–15)*
(12) BALB/c	20/20	5.8 ± 1.2	17 (85%)	10.6 ± 1.6

All mice were injected at 50 d of age intramuscularly in the left thigh with 0.1 ml of 1:2 diluted M-MSV containing $5-8 \times 10^8$ focus forming units per ml when tested in secondary BALB/c mouse embryo cell cultures. The original stock of M-MSV was obtained from Electro-Nucleonics Laboratories, Bethesda as M-MSV-infected BALB-3T3 cells. All virus used in the present experiments was prepared as in ref. 15 as 1 g ml⁻¹ equivalents from M-MSV tumours produced in BALB/c mice, divided into aliquots and stored at -75°C. The virus used was apparently free of LDH-elevating virus, since infected mice showed normal LDH serum levels, measured as in ref. 13. 'Tumours' indicates development of nodules at the injection site. CBA indicates those *nu/nu* and *nu/+* mice which are in CBA/H background. BALB indicates those *nu/nu* and *nu/+* which are in BALB/c background. Sk indicates allogeneic C57BL/6 skin grafts. TG indicates a thymus graft implanted intraperitoneally (i.p.). TDC indicates a thymus within a diffusion chamber implanted i.p. Thy indicates the i.p. injection of 100×10^6 thymocytes from adult *nu/+* BALB mice. All these procedures were performed at 30 d of age. All animals were observed for 50 d after M-MSV injection. Mean regression times were calculated excluding those animals that did not show regression. Asterisk indicates actual regression times. By Mann-Whitney non-parametric tests¹⁶ the differences in regression times between non-overlapping populations are significant.

dependency of these regressions was demonstrated by the effect of thymus grafting in the *nu/nu* mice (groups 4 and 10) in which regression was observed in 92 and 83% respectively of the animals. Conversely, thymuses enclosed in a diffusion chamber were ineffective in supporting regression of the developing tumours (group 5). The small group of *nu/nu* BALB injected with dispersed syngeneic thymocytes (group 11) showed regressions in 2 of 6 instances (33%).

These studies show that the capacity to produce regression of M-MSV-induced tumours has a strict thymus dependency. The exact immunological mechanism by which these regressions are produced is still undefined. Both antibodies^{2,5,8,17} and cells³ can transfer immunity *in vivo* and both T^{9,18,19} and non-T cells^{10,19} as well as normal lymphocytes in the presence of immune sera²⁰ or immune serum and complement²¹ behave as effector mechanisms in inhibition of tumour growth or cell kill *in vitro*. Whatever the mechanism(s), the thymus is required for the generation of such effector products, whether cells or antibodies. Additional factors may, however, be important since considerable differences in tumour growth and regression were observed between different stocks of M-MSV^{2,22}. The inability to restore the capacity to produce tumour regressions in *nu/nu* mice with thymus enclosed within a diffusion chamber, while free thymus grafts were effective, supports our results

30–41% in the *nu/+* and 17–26% in the *nu/nu*. The results in the *nu/+* mice are comparable with those observed by others in early M-MSV lesions²⁴. When these relative values were expressed as absolute number of cells, the differences increased, since the total number of cells per tumour was higher in the *nu/+* mice (mean values 2×10^8 for *nu/nu* and 6×10^8 for *nu/+* mice), however, variability of the cell yield per tumour precludes interpretation without further study. The present evidence can be interpreted as a consequence of impaired granuloma formation and/or the reduced rate of transformation or reduced rate of proliferation of the transformed cells in the nude mice. These differences in cellular composition were also observed in histological sections and are presently under study. This interpretation is also supported by the long latent period for tumour development observed in mice irradiated with 350 r and infected with low doses of M-MSV¹. The proportion of atypical granulomas compared with sarcomas defined histologically was, however, comparable between normal or thymectomised or irradiated mice²³.

The naturally occurring cytotoxic anti-tumour antibodies described in the serum of nude mice²⁵, may also play some role in our observations. These antibodies are complement-dependent and can lyse various tumour cell lines (including two M-MSV lines) and are not detected in *nu/+* mice at any age,

however, the incidence of such antibodies in the sera of individual mice from our colony, both in the BALB/c and CBA/H background, is relatively low, never above 30% of the animals tested at ages ranging from 50 to 120 d (my unpublished results). Of the 12 nudes (BALB/c) in group 8 (Table 1) that were pretyped for presence or absence of such antibodies, three had detectable titres when tested against EL4 cells and M-MSV transformed Swiss and BALB/c cell lines. The two animals with the longest latent period, 21 and 23 d respectively, had no detectable titres. All the *nu/+* tested (group 7, Table 1) had no detectable titres. It is apparent that additional work, especially correlations of *in vivo* and *in vitro* reactivity using this model may solve some of these quandaries. Alternative interpretations such as the possible stimulatory role of the incipient immune response on tumour growth²⁶ or the possible absence of T cells with suppressor activity in the nude mouse²⁷, cannot be excluded.

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¹ Fefer, A., McCoy, J. L., and Glynn, J. P., *Cancer Res*, **27**, 1626 (1967).

² Law, L. W., Ting, R. C., and Stanton, M. F., *J. natn. Cancer Inst*, **40**, 1101 (1968).

³ Fefer, A., McCoy, J. L., and Glynn, J. P., *Cancer Res*, **27**, 2207 (1967).

Erratum

In the article "Two types of resistance to polyene antibiotics in *Candida albicans*" by C. C. HsuChen and D. S. Feingold (*Nature*, **251**, 656, 1974) the following corrections should be made. Page 658, column 1, line 6 should read pore size in cell wall (a molecular sieving effect¹⁶), (3) a temperature. Page 659, line 19 should read and dinitrophenol reduce the effect of the polyenes.

In the legend to Fig. 1, line 4 should read temperature. Only exponentially growing cells were used in these experiments. Washed cells were suspended.

In the legend to Fig. 3, line 2, for 139 read E139A, and the expression in lines 6–8 should have square brackets inserted thus, [(glucose released in the presence of polyene—blank control)]

⁴ Schachat, D. A., Fefer, A., and Moloney, J. B., *Cancer Res*, **28**, 517 (1968).

⁵ Fefer, A., *Cancer Res*, **29**, 2177 (1969).

⁶ Law, L. W., Ting, R. C., and Allison, A. C., *Nature*, **220**, 611 (1968).

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⁸ Bubenik, J., and Turano, A., *Folia Biol*, **14**, 433 (1968).

⁹ Plata, F., Gomard, E., Leclerc, J. C., and Levy, J. P., *J. Immunol*, **111**, 667 (1973).

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¹¹ Wortis, H. H., *Clin. exp. Immunol*, **8**, 305 (1971).

¹² Stutman, O., *Science*, **183**, 534 (1974).

¹³ Riley, V., in *Methods in cancer research*, **4**, (edit. by Busch, H.), 493 (Academic Press, New York, 1968).

¹⁴ Stutman, O., Yunis, E. J., and Good, R. A., *J. exp. Med*, **132**, 601 (1970).

¹⁵ Moloney, J. B., *J. natn. Cancer Inst*, **24**, 933 (1960).

¹⁶ Siegel, S., *Nonparametric statistics for the behavioral sciences* (McGraw-Hill, New York, 1956).

¹⁷ Pearson, G. R., Redmon, L. W., and Bass, L. R., *Cancer Res*, **33**, 171 (1973).

¹⁸ Herberman, R. B., Nunn, N. E., Lavrin, D. H., and Asofsky, R., *J. natn. Cancer Inst*, **51**, 1512 (1973).

¹⁹ Lamon, E. W., Wigzell, H., Klein, E., Anderson, B., and Skurzak, H. M., *J. exp. Med*, **137**, 1472 (1973).

²⁰ Pollack, S., Heppner, G., Brawn, R. J., and Nelson, K., *Int. J. Cancer*, **9**, 316 (1972).

²¹ Tamerius, J. D., and Hellstrom, I., *J. Immunol*, **112**, 1987 (1974).

²² Lavrin, D. H., Herberman, R. B., Nunn, M., and Soares, N., *J. natn. Cancer Inst*, **51**, 1497 (1973).

²³ Stanton, M. F., Law, L. W., and Ting, R. C., *J. natn. Cancer Inst*, **40**, 1113 (1968).

²⁴ Owen, J. J. T., and Seeger, R., *Br. J. Cancer*, **28**, 26 (1973).

²⁵ Martin, W. J., and Martin, S. E., *Nature*, **249**, 564 (1974).

²⁶ Prehn, R. T., *Science*, **176**, 170 (1972).

²⁷ Gershon, R. K., Cohen, P., Hencin, R., and Liebhafner, S. A., *J. Immunol*, **108**, 586 (1972).

(total amount of glucose trapped—blank control)] × 100

In the legend to Fig. 4 the explanation of the symbols does not show clearly the relationship between the neutral lipids and phospholipids. The tabulation below clarifies the situation.

	Neutral lipid of	Phospholipid of
●	E139-A	A1
▲	E139-A	E139-A control
■	E139-A	A3
○	A1	A1 control
▼	A1	E139-A
△	A3	A3 control
□	A3	E139-A

Incorrect symbols were inadvertently used in Table 1 which rendered it meaningless. The correct version is reprinted below.

Table 1 Characterisation of polyene-resistant mutants of *C. albicans*

		Polyene added (μgml^{-1})	Strain					
			E139-A	A1	A2	A3	A4	A7
Growth in liquid medium*	Blank control	0.0	+++	++	+++	++	+++	++
	Amphotericin B	40.0	—	—	—	—	—	—
		20.0	—	+	—	—	—	—
		10.0	—	++	—	++	+	—
		2.5	—	++	—	++	++	—
		0.5	—	++	+++	++	+++	++
	Nystatin	100.0	—	++	—	++	—	—
		50.0	—	++	+++	++	+++	++
		25.0	—	++	+++	++	+++	++
		10.0	+	++	+++	++	+++	++
2.5		+++	++	+++	++	+++	++	
Growth on solid medium†	Nystatin	100.0	—	+	+	+	+	+
	Nystatin + amphotericin B	100.0	—	+	—	+	+	—
		20.0	—	+	—	+	+	—
Colour reaction‡			Greenish blue	Bright yellow	Purplish blue	Bright yellow	Greyish blue	Greyish blue
Doubling time (min)§			65	85	70	97	90	135

* Log phase cells were adjusted to approximately 5×10^4 colony forming units per ml in fresh growth medium, 2 ml of this dilution was added to tubes containing the given polyenes. Dimethyl sulphoxide was present to 1% in all samples including the blank control. After overnight incubation at 37° C, the turbidity was recorded.

† A single colony of the given strain was streaked out on plate containing the given polyenes, and the growth was checked after 24 h of incubation at 37° C. The nystatin ($4,530 \text{ U ml}^{-1}$) and the amphotericin B ($876 \mu\text{g ml}^{-1}$) were gifts from the Squibb Institute of Medical Research.

‡ Liebermann-Burchard reaction used. Total lipid extract of whole cells were used for assay. Preparations from both log and stationary phase cells gave identical results.

§ Growth was monitored turbidimetrically with a Klett-Summerson photoelectric colorimeter (green filter).

reviews

WHEN Maurice Clavelin's *Essay on the Origins and Formation of Classical Mechanics* first appeared in French in 1968, it was widely recognised as a work of much originality, and nothing published since that first appearance has made very serious inroads into his main conclusions. The *Essay* is a long and intensive study of "a transition from one conceptional framework to another, the replacement of one explanatory ideal with another and an unprecedented fusion of reason and reality", and M. Clavelin argues that to evaluate the scope of the revolution "we must try to view Galileo's work from within". He professes to make no systematic reconstruction of the intellectual and social context in which Galilean science evolved, and yet, overlooking these modest protestations, the reader will have no difficulty in extracting just such materials. The book begins with the Aristotelian doctrine of motion—the intrinsic analysis of local motion, the cosmological frame without which local motion (for Aristotle) was inconceivable, and the descriptive analysis of local motion (the classification of it, the evaluation of its magnitude, and resistive force). By emphasising the coherence of the Aristotelian theory, and the interrelations of the cosmos and local motion, M. Clavelin explains how—far from being the heap of inanities some popular histories would have us believe—it could be questioned and extensively qualified by great minds, without collapsing into a pile of rubble. And if that is important to an understanding of Galileo, then the traditions of the 14th century schools of Oxford and Paris was even more so.

In that connection, Galileo for long misled his commentators by lavishing praise on Archimedes at the expense of Aristotle. Of the fact that Galileo's technical language owed much to mediaeval precursors there is no doubt, but how extensive was his debt to them? Like them, Galileo at first tried to relate the increase in speed of a falling body to its spatial position rather than to time (After all, according to Aristotle, motion depended on position.) The mediaeval doctrines were an established part of the teaching he had at Pisa between 1583 and 1586. In his *Juvenilia* Galileo reveals himself as in many respects an Aristotelian, and his early *Treatise on the Latitude of Forms* includes many allusions to the Mertonians and Parisians. Simultaneously

Focusing on Galileo

J. D. North

The Natural Philosophy of Galileo: Essays of the Origins and Formation of Classical Mechanics By M. Clavelin. Pp. xiii+498 (MIT Press, Cambridge, Massachusetts, and London, 1974). \$25.00. *Two New Sciences* Including *Centers of Gravity and Force of Percussion* By Galileo Galilei. Table introduction and notes by Stillman Drake. Pp. xxxix+323 (The University of Wisconsin Press, Madison, Wisconsin, 1974). \$14.00 cloth, \$5.00 paper.

ously with these early writings, however, Galileo was reading Euclid, Archimedes (especially on hydrostatics), and Tartaglia (on projectiles). These works convinced him of the need to replace the textual criticism and qualitative dialectic of the philosophers with a direct and mathematical method. The first taste of the great power of the essentially geometrical method for the analysis of physical problems was evident in the early works *De motu* and *Mechanica*. For the greater *Dialogue and Discourses*, in which the method was exploited far more fully, the world had to wait some decades, and this because from 1602 until his death in 1642 Galileo found a need to link Copernicanism with the mathematical science of the motion of heavy bodies. Into this period, of course, came the telescopic discoveries which so distinguished the Galilean outlook. M. Clavelin reminds us how important was the cosmological component in thought about motion, before Newton's time. Galileo, for instance, was never to abandon the view "that circular motion alone had an actual and real power of indefinite self-conversation". Unlike his precursors, we are told, Galileo was able to treat local motion as a state rather than as a process, and Copernicanism indeed set Galileo the problem of justifying the idea of an orderly world in which motion, not rest, is the normal state of the Earth. His analysis of the Earth's diurnal motion (as in the Second Day of the *Dialogue*) led him to create some of the most significant of his mechanical concepts—such as the idea of an inertial system, the principle of the conservation of uniform motion, and the principle of the composition

of motions. M. Clavelin explains all that beautifully, and at length, steering a middle course between the positivism of Duhem and the Platonism of Koyré. He contrasts the contents of the *Dialogue* with the corresponding parts of the *Discourses*, and at the same time emphasises a "lack of cohesion" in Galileo's mechanical ideas which denied him the opportunity of discussing properly the planetary motions in mechanical terms, and which indeed made impossible the treatment of weight as a force.

Before Professor Drake's work, the last important English translation of the *Discourses* (1638) was prepared by Henry Crew and Alfonso de Salvio in 1914 under the title *Dialogues Concerning Two New Sciences*. Earlier English translations had been published in 1665 and 1730. In the introduction to Drake's new translation, reasons are given for thinking a new version desirable. The changes are not such as to strike the casual reader as very significant, but it is the very fact that they are spelt out at length which gives the new version its value. Take the (Italian) noun *mobile*, for instance, signifying a tangible and heavy moving object near the Earth's surface. This is now rendered by the English 'moveable', in contradistinction to the adjective 'movable'. Crew and De Salvio translated *mobile* to mean 'particle', to which objection is now made on the somewhat curious grounds that "the modern physical particle is essentially devoid of weight". As another example, Professor Drake dislikes the introduction of the mediaeval concept of "mean speed" into the statement of Galileo's basic first theorem on accelerated motion, in place of "one-half the final speed". Whatever the reader's feelings over these subtleties, it is undeniable that the new version must now replace the others. It includes original Galileo works which were published posthumously in the form of a dialogue on the force of percussion (intended for the 1638 edition, but not finished to Galileo's satisfaction), and Galileo's work on centres of gravity. Professor Drake, whose reputation as a Galileo scholar is well known, adds some useful notes, as well as a bibliography of the principal editions and translations of the *Two New Sciences*. The new version, like the original Leyden (Elzevirs) edition, is very attractively designed and printed, and not unduly expensive. □

Management of natural systems

Genetics of Forest Ecosystems By Klaus Stern and Laurence Roche Pp 330 (Chapman and Hall London, Springer Berlin and New York, 1974) £12 25

In his preface Roche states that forest ecosystems are a rich source of information on ecological genetics but that published evidence has not yet significantly penetrated the botanical literature. Little exception could be taken to the first part of that statement, and the book, itself a rich source of information, goes some way towards correcting the imbalance mentioned in the latter part. But according to the introductory remarks by Stern the book aims to be more than a catalogue of information on stable and transitory forest ecosystems. Stern assumes that forest ecologists and breeders have been more concerned than agronomists with the inner mechanisms of ecosystems and that only by knowing these mechanisms could the former group hope to improve the economics of forestry. Accepting that this is true and that ecologists and breeders significantly affect forestry practice, then discussion of the state of man's knowledge of these mechanisms, which form an important part of the book, is a valuable contribution to the intelligent management of the world's forest resources.

The main subjects—ecological niches, adaptations, genetic systems, adaptive strategies and forest ecosystems—are approached in a similar manner throughout. First, formal definitions are stated and discussed and that is followed by a consideration of mathematical concepts and their limitations and applicability. Finally, examples are given, mainly from the forest ecosystem, to illustrate and complement the previous discussions. The use of the concepts of systems theory, games strategy and cybernetics will provide difficult reading to the uninitiated. Such difficulties are compounded by the odd misprint, the occasional grammatical peculiarities, and a style of writing which may well be a consequence of translation from the original German.

In a book of this scope it is always possible to find something to criticise but I must say that I thought that the chapter on genetic systems of forest trees species placed undue emphasis on selfing, whereas the reader was referred elsewhere for information on other aspects. And there is much space devoted to Fowler's work on *Pinus resinosa* but his conclusions are reported erroneously in the text. On the other hand, there is much of which I approved, such as the discussion of the

tropical ecosystem, which emphasises and contrasts the ideas of Federov and Ashton.

The final chapter, contributed by Roche, is a review of man's effect on the ecosystem. It is proposed in the introduction that this chapter could well have been placed in a separate book. Had it been placed at the beginning of this book, however, its contents would have encouraged those whose activities affect the forest ecosystem to read the following chapters.

Ian R. Brown

Viral diseases

Slow Virus Diseases Edited by John Hotchin Pp xvii+372 (Karger AG Basel, London and New York, 1974) £19 80, \$49 75

THE title of this book is misleading for the text is concerned mainly with persistent viral infections. 'Slow' infections constitute only a small and unrepresentative group. Seventy pages describe the arena virus group of which LCM virus is the prototype. In contrast, the 'slow' virus diseases are described less adequately. Thirty pages outline reasons for viral persistence, and examples of diseases of man and animals in which persistent virus is considered important in pathogenesis are written about with varying clarity by 27 specialists. All in all, the book is a useful introduction to a developing but not well-defined area of medicine. John T. Stamp

Plants beneath the rising Sun

The Flora and Vegetation of Japan Edited by M. Numata Pp x+294 (Kodansha Tokyo, Elsevier Scientific London and New York, 1974) Dfl80, \$30 80

SINCE 1950, the Japanese have achieved an international reputation for the originality and importance of their contribution to quantitative studies in plant ecology. The analysis by Monsi and Saeki of the attenuation of solar radiation intercepted by herbaceous vegetation, the application by Hozumi of allometric methods to the estimation of growth of forest stands, and the studies by Kira and his associates of intraspecific competition are all examples of work which has attracted world-wide attention. Recognition of the significance of this work has been facilitated by publication in English and German. In contrast, most of the literature on the vegetation of Japan

has been published in Japanese so that it remains relatively inaccessible. Now a concise guide is available in English and it provides an excellent account of one of the most intensively studied and interesting vegetational regions of the world.

Modern Japan extends from the northern coast of Hokkaido (latitude 46° N) to the southernmost of the Ryukyu islands which is almost on the Tropic of Cancer, a distance of over 3,000 km along the great archipelago which stretches from Kamchatka and the Kuril Islands in the north to Formosa (Taiwan) and the Philippines in the south. During the climatic vicissitudes of the Pleistocene, the continuity of the archipelago seems to have allowed much of the Eocene flora to survive by oscillating from north to south and back. Consequently, the flora of Japan is extraordinarily rich, with almost 4,000 vascular plants, including many endemics and relict species of the Arcto-Tertiary flora, and a great wealth of woody species.

Small areas of sub-tropical vegetation occur on the Ryukyu islands but the original vegetation on the main islands ranges from warm temperate forests, comprising largely evergreen broad-leaved species in Kyushu, Shikoku and southern Honshu, to cool temperate forests, characterised by beech (*Fagus crenata*) and representatives of several familiar temperate genera such as *Acer* and *Prunus*, in northern Honshu. Subarctic coniferous forests of *Abies sachalinensis* and *Picea* spp. are present on the mountains of Honshu and descend to sea-level in Hokkaido. This latitudinal zonation is accentuated by the warm Tsushima and Kuro-shio currents which originate in the tropics and have a large influence on the climate of the southern islands but which fail to reach Hokkaido.

Much of the land which would naturally carry forest has been modified greatly and is now occupied by grassland or vegetation dominated by *Pteridium aquilinum*. There is a wide variety of coastal vegetation and extensive regions of subalpine and alpine vegetation, in which a majority of the genera are those important in Europe. The final section is devoted to an account of the vegetation of active volcanoes and includes some remarkable illustrations of recent lava flows and pumice deposits now covered by forests.

The book provides a clear and fascinating account of Japanese vegetation and of the many geographical and ecological problems presented by its complexity and its considerable modification by man. It is well written, well balanced in its presentation and has numerous illustrations which are uniformly of high quality. C. D. Pigott

nature

January 17, 1975

Should we leave it to the mystics?

THE French postal strike delayed until recently the arrival of the latest of one of UNESCO's more interesting periodicals, *Impact of Science on Society*. There are several journals attempting to fill the role of spanning the gaps between science, policymakers, educationalists and the public, and all of them, one suspects, suffer from the same problem: the scientist feels he should read them, but never quite finds time.

Impact (its long title sounds a little dull, its short one suggests it should be on the bookstalls along with *Probe*, *Thrust*, *Health* and . . . *Nature*) is always worth devoting time to, and this most recent issue, on the parasciences, is no exception. A dozen writers provide essays on subjects ranging from the design for a spaceship reported in the Book of the prophet Ezekiel to vision through the fingers. The whole is elegantly introduced through an essay (adapted from the *The Roots of Coincidence*) by Arthur Koestler.

One of the persistent complaints of those who work in fields outside orthodox science is that they cannot get scientists to take them seriously—although one must note that of 48 past presidents of the Society for Psychical Research, nine were Fellows of the Royal Society. And yet there are some, both scientists and parascientists, who believe that the two fields represent opposite sides of the coin and scientists should not be brought in to attempt to explain what is inexplicable in their terms (in much the same way that science and religion seem, on the whole, to have kept each other at a respectful arms-length). It is thus a matter of some interest to see what parascience says when given a highly respectable platform from which to speak to the establishment.

The result is somewhat disappointing. Part of this, no doubt, stems from the necessary variability produced in bringing together authors from many nations. But part of the disappointment is that, with the exception of Koestler, none of the proponents of the parasciences really gets down to the serious business of trying to persuade the reader that there is something in it.

Koestler is an exception because he can see the strange side of the modern physicist's perception of nature and he can play on it to create a certain malaise in the reader's mind. Time reversals, anti-matter, a hundred or more elementary particles—scientists can accept these, but still choke on the thought of extrasensory perception, let alone psychokinesis. It's a good though certainly not a clinching argument, but much of what follows in the journal demonstrates why it is that scientists are still sceptical. Practitioners either indulge in the production of wide-ranging samplers of superficial and inadequately reported observations, or plunge into extended deep speculation without so much as a glance at the reader to see if he

needs any convincing even to take the first step.

Yet for all this there are some good things, particularly where science and technology are allowed to do the explaining. Kirlian photography, the observation of corona discharges, is well dealt with by Rudolph Guzik, who makes it clear that a very complex (and in a sense unpredictable) phenomenon can be understood within the confines of 'normal science' provided that mystics, using words like 'life force' and 'psionic aura' do not enshroud the whole field in nonsenses and drive away those who could put it on a rational footing. One suspects that this is a paradigm for much of what exists in and beyond the fringe of science. There are many things which in the past were deemed not 'normal', that is not common-place experience. Among these could be included eclipses, earthquakes and mirages, and all of these have yielded to rational study. Nor is science reckoned to have failed because some of these phenomena are irregular and unpredictable as yet. Other 'not-normal' phenomena, being less easy to master scientifically and more susceptible to the attentions of quacks and tricksters, have now receded into a world of the half-light, and the scientist wishing to investigate has first to discern whether he is being taken for a ride by the practitioner. He may rather be taken for a ride by his own senses, and this indisputably is a subject worthy of research; a scientist may be able to satisfy himself that the practitioner is not consciously deceiving but be unqualified to spot his own perceptual errors. For a start he should be suspicious of any phenomena which require darkened rooms or his ability to absorb a wide variety of happenings and pronounce on the normality or otherwise of one of these—not necessarily the one he was concentrating on.

There may well remain, however, things worthy of our attention—a sort of dry residue. The parasciences may not yet have unearthed much for which there is a cast-iron case for scientific explanation, but there is ample material crying out for verification, and the lessons learnt over the last few years should have improved techniques and procedures for getting to the heart of the deception/reality question. Now, surely, is the time for scientists in large numbers to take a more practical interest in these obstinate issues. If we fail to do so, parascience will recede further into the mystic's world and will take with it much of the public's sympathy.

Investigation in the para-world is neither easy nor always agreeable. Public interest, however, demands, rightly or wrongly, that scientists come to grips with the propositions being made. And if scientists won't come in with rational attitudes and, where necessary, rational explanations, they can hardly be surprised if the forces of irrationalism take over. □

Any old Fe₂, Cu, Al, Pb, Zn, glass, paper. . . ?

The future availability and price of key raw materials, placed in doubt by the events of the past year, have emphasised the need to conserve by recycling. Attitudes in the UK are reflected in the following three articles. The first is by Christine Thomas, of Friends of the Earth.

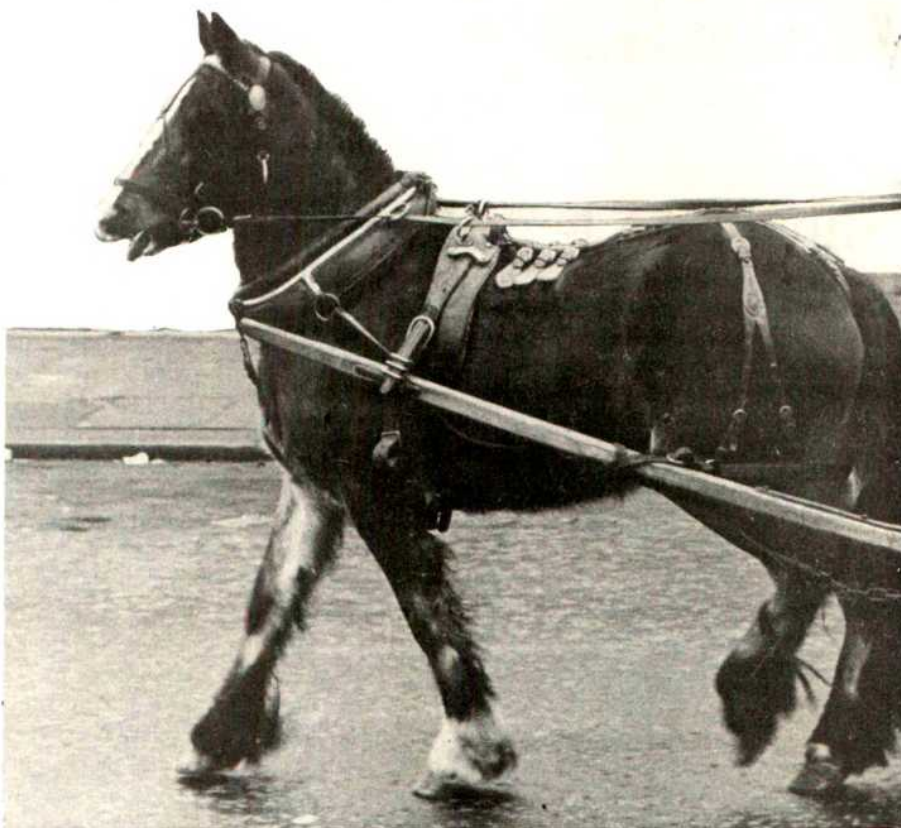
IN modern times, industrial societies have been characterised by an attitude to the use of resources which earlier societies necessarily regarded as untenable. The difference in attitudes was born of a different appreciation of resource availability: in recent years industrialised regions have evolved a sophisticated system of resource-monopolisation and hence cost-control which enabled them for all practical purposes to regard raw materials as infinite or at least theirs for the taking.

This system and the attitudes which it bestowed are now breaking down for two reasons. First, there has emerged a greater maturity of view shared by many of the countries described alternatively as 'developing' or 'emergent', which have important raw material reserves. Second, the real resource scarcity predicted for certain commodities has begun to bite and has been reflected in the rising prices of raw materials.

It is therefore self-evident that the more efficient use of raw materials in 'developed' countries should become an important 'development' goal. Britain, a country more heavily dependent than most on the import of essential raw materials, can no longer afford to discard over 100 million tonnes of waste every year. Even if more efficient recycling systems and policies of re-use were instituted, Britain would still be dependent on raw material imports.

Recycling of industrial products is one option that can lead to a thrifter use of resources. By attempting to approximate to natural cycles, such as those for carbon and nitrogen, where no material is lost from the system, recycling can partially close the loop of industrial production. By doing so this decreases both the production of waste and the extraction of virgin raw materials associated with a given level of materials consumption.

Current practice though shows these loops to be far from closed. The table below illustrates the present use of reclaimed materials in Britain. The figures in the first column are deceiving as they include the use of 'new' scrap or manufacturers' scrap. More telling is the percentage of 'old' or 'post-consumer' scrap recovered.



Material	% (by weight) of total production accounted for by:	
	All scrap	Old scrap
Iron and steel	52	10
Copper	40	13
Aluminium	30	—
Zinc	25	—
Lead	65	—
Glass containers	22	3
Paper	43	15-30

Comparison between the energy requirements for a number of metals produced from secondary materials and from their ores show that for the former the energy needs are invariably smaller. Often dramatically so, as with aluminium where the energy costs of its production from bauxite are 30 times that for its production from scrap. Recycling paper also offers potential energy savings, as production of fibreboard from virgin pulp requires about twice the amount of energy compared with its production from waste paper.

The British Steel Corporation accrue considerable savings through their recycling efforts. They used 19 million tonnes of scrap steel last year, nearly half their raw material requirements and with a 30% saving in energy associated with processing scrap this resulted in almost 80,000 kWh (thermal) saved.

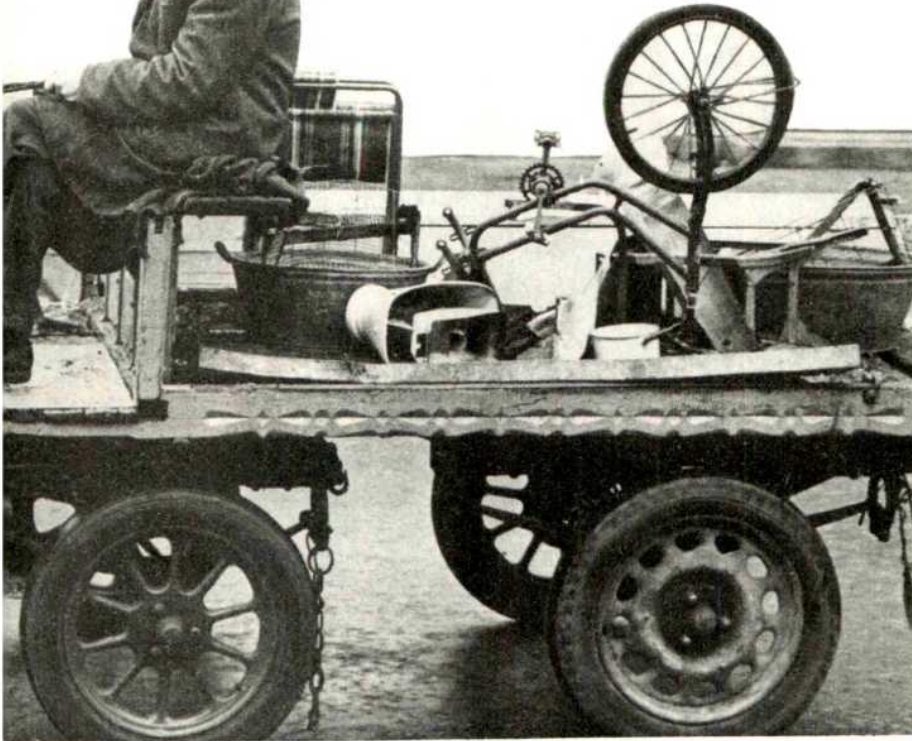
Since it would seem desirable on many accounts to maximise our recycling effort, why is current performance so low? Basically it is a question

of economics. In many cases it does not pay the individual firm to recycle, even though it would be of benefit to the country as a whole. A situation therefore arises in a free market, in which industry can externalise the social and environmental cost of production, creating a situation where virgin material use does not reflect its true cost to society. Thus recycling suffers unreasonably in competition with raw materials.

One controlling factor which is often overlooked and which is of great importance in deciding what is a desirable level of recycling is energy. In many cases recycling would not be justified where the energy costs of recovering a material far exceed that of initial production. Generally this is not the case, but mixing materials together, particularly in small proportions, makes it energy expensive to reverse the process and recover one material.

An additional constraint on the achievements of recycling is imposed by our attempts to continue growth in the production of consumer goods. In a 'growth' situation, reclaimed material arising from the previous year's production cannot by definition meet this year's demand. And in a situation of exponential growth the gap between the total supply of materials required to meet demand and that met by recycled products will continue to diverge.

The front rank of the scrap metal trade, where an infrastructure of merchants has been built up over many years to feed metals back to the smelters.
Picture by John Rigby.



John Newell reports on a recycling system which is being operated at a government laboratory.

THE automated pilot rubbish sorting line is now working at the Department of Industry's Warren Spring Laboratory. And most of the data concerning the total municipal and industrial waste produced by the county of Oxfordshire has now been assimilated in a project at the same laboratory which is intended to estimate how much of the raw materials needs of such a typical area could be met by a wasteplex, a single, central, all-purpose recycling centre. Great interest has already been shown in the Warren Spring Waste Materials Exchange, which will act as an information service providing regular quarterly bulletins, shopping lists of waste products which could be just what are needed as raw materials by someone else. Although the Warren Spring Laboratory is now spending at least four times as much as they were five years ago on recycling engineering, their resources are still limited when compared with the USA. The members of the team, which has been trying—and now succeeding it seems—to interest industry in new recycling technology developed at the laboratory, can be excused some irritation with accusations in the media that they are partly responsible for sluggish British

interest in moving away from tipping and towards recycling of solid and other wastes.

The sorting line now occupies about half a hangar-sized shed. It is fed by six tons at a time of raw rubbish from Stevenage dustcarts. The rubbish goes through a bag burster and then a rotating drum, which sorts it through three successive sieves with increasingly large holes. The various conveyors coming off this take their loads past magnets, low powered fans, a water bath to separate light from heavy constituents, and devices where rubbish is flung off high speed conveyors against angle plates, or drums rotating in the opposite direction, to make use of differences in the coefficients of friction and resilience.

Out of the other end, or rather ends, come several potentially valuable by-products. Three grades of reusable paper, two already pronounced as suitable for board making by commercial board mills, one suitable for high grade fuel; clean tin cans for remelting; vegetable matter for composting or conceivably for animal feed, coarse rags for floor covering materials and fine particle materials for soil conditioning.

Warren Spring has also developed means for separating glass to a very high degree of purity because, although there is little commercial future for recycled glass in high grade uses at

present, the position may change, and recent developments in glass fibre manufacture make it likely that this will soon become a large scale outlet for recycled glass.

The lowest grade, dirty, wet mixtures of plastic, paper and other organic unmentionables could at least provide heat, if distilled in a pyrolysis plant set up on a wasteplex site. Warren Spring finalised its designs for a pyrolysis plant earlier this year, after some years of research, and are now waiting for the go-ahead to build a commercial version for a local authority. It has also had fully developed for some time an ingenious fluidised-bed technique for separating different non-ferrous metals.

As a result of talks now in progress, Warren Spring hopes that a first rubbish recycling line, probably using not just a few elements but most or all of the different sorting techniques it has developed, will be built for a local authority somewhere in Britain within 12 months. There will probably be no need to scale up since the existing plant can cope with 50 tons every 24 hours—the equivalent of the rubbish output of a city of about 50,000 people.

Unlike rivals, the Warren Spring system keeps rubbish in large lumps as far along the line as possible. Shredding is a late stage. This prevents the transfer of dirt to paper and tin cans that are being squashed inwards into their contents, and generally makes sorting, especially of glass, much easier. The Warren Spring line uses relatively few low powered air fans, which cuts running costs and does away with the need for cyclone air purifiers.

Some 25,000 to 30,000 companies are being approached during the setting up of the waste materials exchange scheme, in which Warren Spring hopes to supplement the existing communication lines of this kind with their quarterly catalogue, which will list the more *outré* waste products, including a good many normally considered toxic but still potentially recoverable. One example has already been the reclaiming of seeds, surplus to requirement which had been given a dressing of a mercuric fungicide; this enabled them to be conveniently recycled into an adhesive for wallpaper, also requiring a fungicide.

Full scale wasteplexes including, as well as pyrolysis plants and sorting lines, specialised centres like that at Pontypool for the reclamation of toxic wastes, and plants for de-inking news print, are perhaps 10 or 15 years away in Britain. But the Oxfordshire study which began in August will soon provide detailed guidelines for such projects which are adaptable for a wide range of districts—Oxfordshire was chosen because of its many and various small industries and life styles.

T. S. McRoberts, Director of the Wolfson Recycle Unit, Queen Mary College, London, describes the background to the unit's work.

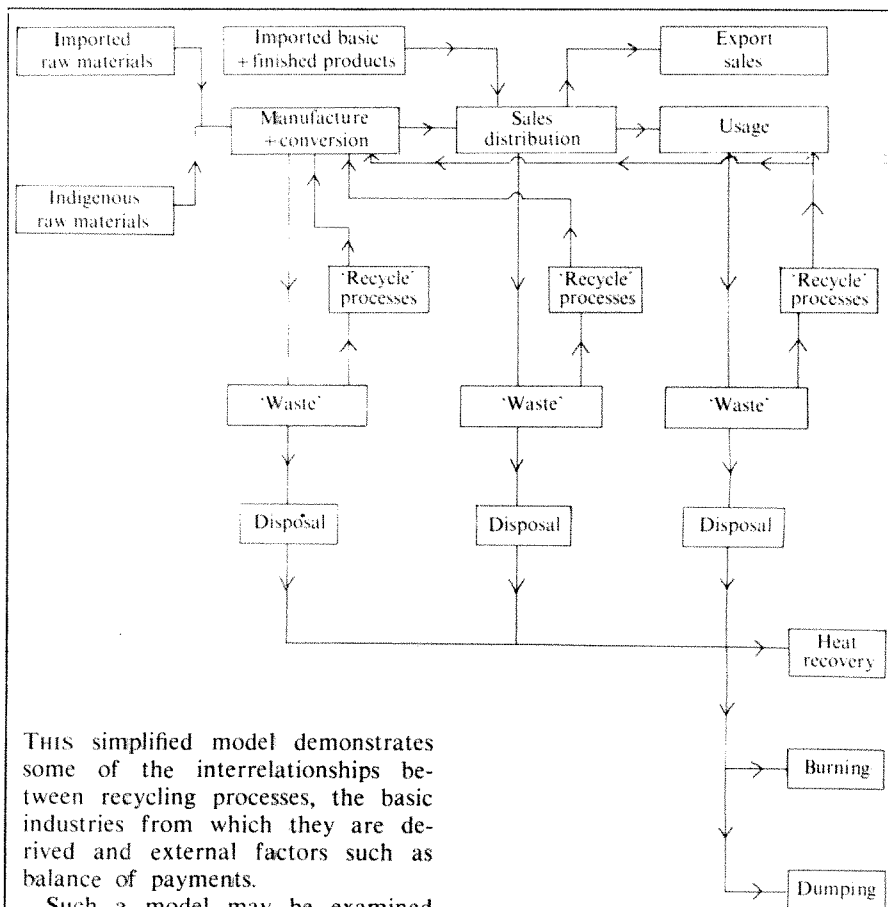
IN ANY study of recycling a great deal can be learnt from what industry is already doing. Many industries already carry out recycling in some form. The chemical industry operates large scale polymerisation plants where the economics of manufacture depends critically on the recycling of monomer. The efficient converter of plastics recovers and reuses offcuts and rejects from moulding operations. The UK paper and paperboard industry finds almost half of its feedstock in waste paper. Yet the bulk of plastics are consigned to municipal dumps after their first use and only about a third of paper is returned to mills for reconstitution and reuse.

Recycling of an obviously profitable nature is already being practised. The most notable example is the scrap metal trade, where an infrastructure of small and large merchants has been built up over many years to feed ferrous and non-ferrous metals back to the smelters. Only the more easily available and profitable sources are tapped, however, and it is estimated that 1 million tonnes of metal are discarded annually into domestic dustbins.

A major obstacle to the reuse of materials has been the cyclical nature of many key industries. At times when business is in the doldrums and mainstream plants are underloaded, prices of products from virgin materials are depressed close to marginal manufacturing costs and the waste recovery industry is immediately jeopardised. Among such industries are the plastics and paper industries, which are the subject of a technical and economic study by our unit.

Both the plastics and paper industries are now entering a recession. The comparatively new plastics recovery industry is under severe economic strain and waste paper merchants have large amounts of expensive working capital tied up in stocks which are proving difficult to sell.

While the waste recovery industry is preoccupied mainly with its own efficiency and profitability, the nation as a whole has a balance of payments crisis linked to heavy importation of energy in the form of oil. The UK imports most of its paper requirements in the form of pulp or finished paper; for example domestic wood pulp accounts for only one-sixth of its wood pulp requirements. The plastics industry depends almost entirely on feedstocks from oil, all of which is at present imported. It is obviously important from a national viewpoint that



THIS simplified model demonstrates some of the interrelationships between recycling processes, the basic industries from which they are derived and external factors such as balance of payments.

Such a model may be examined with several different objectives in mind, such as (1) maximisation of profits to the companies involved; (2) minimisation of energy use; (3) maximisation of exports; (4) minimisation of imports; (5) maximum contribution to balance of payments.

The profitability of a recycling process may be affected greatly by the actions of a government preoccupied by items (2) to (5) as well as by the demand and supply situation for products from virgin material.

A macro model of the type outlined above is a useful accompaniment to any microstudies, since it enables the effect of changes in external factors to be predicted.

the maximum amount of worthwhile recycling of both paper and plastics should take place. The future of recycling in these industries will depend to some extent on a successful formula being found for mitigating the worst effects of economic fluctuations in the primary industries.

The technology of recycling is influenced by the fact that both paper fibres and plastics materials deteriorate in quality in the course of both processing and reprocessing. Each time paper or paperboard is recycled its fibres become shorter and therefore less satisfactory for paper and paperboard products requiring strength. Although this does not appear yet as a limiting factor in recycling, it may well have to be

The basic parameters describing such a model are interrelated and capable of mathematical treatment. Constraints, such as limitation of size of market, can be expressed as inequalities, and mathematical programming can be used to choose between competing procedures or processes on the basis of maximising functions such as profit or minimising functions such as energy and external payments. This model approach can be extended to company level where profit is the main consideration in deciding between alternative processes. It can be used also to guide research into the potentially most rewarding areas.

taken into account as more and more paper is recovered.

The problem of deterioration in quality and performance is much more serious in the case of plastics. Of the two major groups, thermosets and thermoplastics, only the latter can be considered seriously for recycling purposes. The largest outlet for thermoplastics is in packaging, which absorbs 35-40% of production and most of which appears as waste within weeks.

One would prefer to recycle a material back to the use from which it came, but if the material is degraded on reprocessing, so that it is not acceptable for its original use, a crucial question will be the size of the market for the degraded material. □

international news

A TOP-LEVEL advisory committee has told NASA that, unless the agency's dismal budgetary outlook improves, it should take a close look at its plans for planetary research and perhaps even drop or defer two important missions to the outer planets in the late 1970s. The panel makes that suggestion reluctantly because it believes that the expensive planetary flights could squeeze out cheaper, but equally important, missions.

The recommendation, contained in a report prepared by the Space Science Board (SSB) of the National Academy of Sciences, is one of several suggestions about what should be the priorities in space research in the coming years. The report is likely to be highly influential in shaping NASA's science programmes because it was produced by a panel of distinguished (read powerful) astronomers and space scientists with the help of a large number of university researchers. It will be published early in February by the academy.

Starting from the assumption that NASA's space science budget is unlikely to grow much, if at all, in the next few years—an assumption which is borne out by recent trends—the SSB has recommended what it calls a balanced programme by assigning priorities to missions which have already been approved by Congress and, more important, to missions which are still in the planning stage. Although the report says nothing about manned space flight or applications satellites, it assumes that the space shuttle will be developed to put large payloads into Earth orbit.

As far as approved projects are concerned, the SSB strongly supports the High Energy Astronomy Observatories, the Pioneer Venus project and the Mariner Jupiter-Saturn mission, all of which have been reduced in scope and delayed by budgetary restraints. Those three missions, the report says, "represent the next opportunities for important advances in space astronomy and in planetary and lunar exploration." The Pioneer-Venus programme, with the launch scheduled for 1978, is however, already in danger of being delayed by several months as a result of budget cuts proposed by President Ford in November last year, Congress has yet to act on those proposals.

As for projects which are still in the planning stage, the SSB recommends

A blueprint for space research

by Colin Norman, Washington

that a start should be made next year on the Large Space Telescope (LST). An optical telescope which would be flown on one of the early shuttle launches, the LST would, according to the SSB, be "an exciting major step in the history of observational astronomy, observational cosmology and the understanding of the Universe." But it would also be extremely expensive. Consequently, the report suggests that NASA should consider a rather less ambitious LST than it had originally planned to develop.

In short, the LST has been envisaged as a 3-m instrument, but the SSB suggests that it should be reduced to 2.4 m. Even that would, however, be able to detect stars ten times fainter than is possible using ground-based instruments, and it would also have a resolution ten times better. Given the bleak budgetary climate, NASA will probably be only too pleased to take the cut-rate option.

The LST would be the only new start for the next fiscal year, but the SSB suggests that NASA should begin developing hardware for eight other new missions in the fiscal year after that. They are a satellite which would be placed in polar orbit around the Moon, analysis of data from the 1976 Viking Mars Lander mission and planning of further Mars exploration, a spacecraft which would be sent into orbit around Jupiter and which would send probes into Jupiter's atmosphere, a spacecraft which would swing past Jupiter and travel on to Uranus, an orbiting telescope to take advantage of maximum solar activity in the early 1980s, a satellite for infrared astronomy, a gamma-ray satellite and finally, a spacecraft for electrodynamic studies.

Unfortunately, however, if all those projects are given the green light in the 1977 fiscal year, expenditures in subsequent years would probably break NASA's budgetary ceiling. The SSB therefore suggests that if NASA's

budget fails to increase, the agency should either delay developing and launching the Jupiter orbiter for four years or it should scrap plans for the mission to Jupiter and Uranus.

Although both of those projects have considerable support in the scientific community, the SSB says that if they are approved at the expense of high priority programmes in other fields, the space science programme would be unbalanced. Because the launch date of the Jupiter-Uranus spacecraft is tied to a rare alignment of the outer planets, that mission could not be delayed.

Between 1978 and 1982, the SSB recommends that NASA should begin work on a variety of other projects, including an imaging radar which would be placed in orbit around Venus, orbiters around Jupiter, Mercury and Saturn, a spacecraft to intercept the comet Encke, another infrared telescope, a 1.2 m orbiting X-ray telescope, and possibly a spacecraft which would travel out of the ecliptic plane.

The report makes clear that there is a difference of opinion among planetary scientists about the exploration of Mars after the 1976 Viking lander mission. The report recommends that a long term goal should be to bring samples of the surface of Mars back to Earth for analysis and inspection, but that the next stage of exploration should be to place a spacecraft in polar orbit around the planet, and to follow that with a probe and hard lander in the early 1980s. An SSB subcommittee on planetary research urged, however, that existing spacecraft should be modified so that another Viking lander could be despatched to Mars in 1981 if the 1976 mission produces particularly exciting results. But the SSB report said that keeping such an option open would be too expensive. "we see no missions that should be displaced to meet such option costs", the report states.

The missions recommended by the SSB would keep NASA's space science budget essentially the same in terms of purchasing power over the next six or seven years, but with inflation running at its present rate and President Ford preaching fiscal restraint, it remains to be seen whether even that modest expectation will prove to be too optimistic. There should be some clues when the Administration publishes its 1976 budget recommendations on February 3. □

Canada adopts new nuclear safeguards

from David Spurgeon, Ottawa

STUNG into reconsidering its nuclear export policy by India's explosion of a nuclear device last year, Canada has announced that more stringent safeguards will now be applied to sales abroad of its nuclear technology, facilities and material. But, instead of calling for a complete ban on nuclear sales—as it once considered doing during a seven-month policy review—Canada has decided actually to encourage such sales provided new safeguards are met.

The decision has considerable importance. The Indian explosion (achieved with the unintended aid of a Canadian research reactor and personnel training programme) embarrassed government officials here just at a time when the CANDU reactor's remarkable success at home was beginning to pay off in export sales. By stirring Canadian consciences with the threat of further nuclear weapons proliferation, the Indian incident threatened to frustrate one of the country's few successful forays into high technology just when the superiority of CANDU was being proved.

To some observers, the prospect of Canada failing fully to exploit commercially a successful technology, after the expensive research and development had been accomplished with government funds, seemed balefully familiar.

The government's rationale for its new policy, announced on December 20 by the Minister of Energy, Mines and Resources, Donald S. Macdonald, was thus to place in balance the need for foreign trade of the Canadian economy, the need for cheap electrical power of developing countries at a time of world-wide oil shortage, and Canada's responsibility for ensuring that her nuclear resources do not contribute to the proliferation of nuclear weapons.

"The provisions, to be administered by the International Atomic Energy Agency, or through appropriate alternative procedures meeting the requirements of the Treaty on the Non-Proliferation of Nuclear Weapons", said Mr Macdonald, "will cover all nuclear facilities and equipment supplied by Canada for the life of those facilities and equipment."

"They will cover all nuclear facilities and equipment using Canadian-supplied technology. They will cover all nuclear material—uranium, thorium, plutonium, heavy water—supplied by Canada, and future generations of fissile material produced from or with these materials. They will cover all nuclear materials, whatever their origin, produced or processed in

facilities supplied by Canada."

"Most importantly, all safeguards arrangements will contain binding assurance that Canadian-supplied nuclear material, equipment and technology will not be used to produce a nuclear explosives device, whether the development of such a device be stated to be for peaceful purposes or not."

This latter remark was a reference to the Indian case, in which that country claimed not to have violated previous agreements with Canada because her nuclear explosive was for "peaceful purposes"—an interpretation never agreed to by Canada but now explicitly excluded for the future.

Potential Canadian exporters of nuclear material, equipment or technology were advised that in future they will have to ascertain from the government that there are no safeguards or impediments to sales before making offers of supply.

Having outlined the safeguards, Mr Macdonald said that, "to ensure that Canadians will enjoy the economic gains from sales abroad, the government will encourage the supply from Canada of major high technology components and services." It would also seek domestically to "establish a cooperative approach of preference (among the provinces) for Canadian material equipment and services."

The Canadian industry at present has the capacity to produce at least three nuclear reactor power systems a year, the minister said. Domestic requirements will average four units a year over the remainder of the decade, and exports could add at least one additional unit a year. Nearly \$100 million in capital investment has already been committed or planned by the private sector to expand capacity, and future demands will require perhaps \$100 million more. The federal Department of Industry, Trade and Commerce will see if it can help provide it through incentive programmes.

Meanwhile, the government has lifted the freeze put on nuclear exports after India's explosion and has authorised Atomic Energy of Canada Limited to negotiate a number of foreign sales and agreements, provided that they comply with the new safeguards.

These include agreements with the United Kingdom covering CANDU/SGHWR technological exchanges and heavy water supplies, a licensing agreement to supply CANDUs to Italy, goods and services estimated at \$150 million for a second 600-MW CANDU power station for Argentina, two 600-MW CANDUs for Iran—possibly four, one reactor for Korea, the nuclear part of a CANDU for Denmark, and technology agreements with Rumania.

Opposition parties have said that the new policy does not go far enough to prevent the spread of nuclear weapons. James Balfour, of the Progressive Conservatives, said nuclear agreements should not be signed unless the country involved allowed inspection of all its nuclear facilities, not just those acquired from Canada. This would prevent countries from simply copying a CANDU and using it to produce plutonium.

But Mr Macdonald claimed that the new policy makes Canada's safeguards standards higher than those of its competitors—and only time will tell if that will hurt sales. He said that International Atomic Energy Agency safeguards do not cover technology or prevent the use of nuclear materials for peaceful purposes, as Canada's now do.

In the last analysis, of course, everyone recognises that no form of international agreement will guarantee non-proliferation of nuclear weapons, that depends upon each nation's willingness to abide by the agreements. Mr Macdonald admitted as much outside the Commons after his announcement. And if trade partners with Canada decide to break their agreements, he said, Canada will have no recourse but to embargo future nuclear sales, as was done with India. □

Useful, unsurprising

THERE are no surprises in the recent report on energy conservation prepared by the National Economic Development Office (*Energy Conservation in the United Kingdom*, HMSO, £3.40) but a useful collection of relevant facts and figures which have been lacking in some other surveys.

As expected the report pinpoints domestic and office heating as the main areas in which savings can be made without too much social upheaval, and suggests a comprehensive analysis of the type of fuel and energy supply which would give the most efficient use of primary energy. The facts show that the net energy supply to the domestic market increased very little from 1960 to 1972, although the pattern of fuel use changed drastically. About 70% of this energy goes into space heating, and as the number of homes is steadily increasing and the population is changing to include a greater number of elderly people who will require higher living temperatures, the greatest savings in energy demand can be made here by a much more widespread use of proper insulation. An energy saving of 22% for a new three-bedroomed, semi-detached, centrally heated house could be achieved at an extra cost of a few hundred pounds a house. □

THE long awaited verdict on whether or not the artificial sweetener saccharin causes bladder cancers in rats may still be several years away. Last week, a committee of the National Academy of Sciences, which had been examining the evidence for two and a half years, said that there is not enough reliable data to state that saccharin is or is not a carcinogen. Consequently, the Food and Drug Administration (FDA) announced that it will do some more tests and continue to freeze consumption of the sweetener at 1972 levels.

The academy's report, which presents a detailed analysis of the history and chemistry of saccharin as well as a searching critique of the carcinogenicity tests, leads, however, to two disturbing conclusions. The first is that there has been a scandalous lack of study by the FDA of a chemical which is consumed by about 12 million people in the United States, at the rate of some five million pounds a year. And the second is that most of the saccharin produced in the United States is contaminated by an impurity, *o*-toluene-sulphonamide (OTS), which could itself be a carcinogen. The latter possibility may not only have distorted carcinogenicity tests on saccharin, but it also raises the question of why the FDA has not taken steps to determine the biological significance of OTS and, if necessary, to ensure that the sweetener is manufactured in such a way that the impurity is kept out.

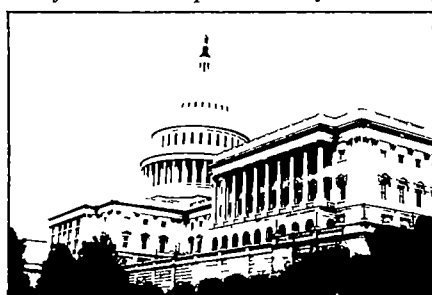
If OTS is, indeed, a carcinogen when fed to animals, the so-called Delaney amendment to the Food and Drug Laws would require that saccharin contaminated by OTS must be banned from the market. Saccharin is manufactured by two commercial processes, the most widely used of which produced OTS impurities at the level of several hundred parts per million, whereas the other results in contamination of only a few parts per million.

There have been two principal studies which raised fears that saccharin may cause bladder tumours in rats. One was carried out by the FDA itself and the other by the Wisconsin Alumni Research Foundation (WARF)—a laboratory supported by the sugar industry. Both studies revealed increases in the incidence of bladder tumours in rats fed saccharin at doses of 7.5% and 5% of the daily diet. The sweetener was administered to pregnant rats so that their offspring were exposed before birth, through the mother's milk and subsequently in their own diets.

The academy committee points out, however, that because the rats were also getting OTS "at very large dose levels", it is "impossible to say whether

saccharin itself was the carcinogen". The report also raises the question of whether bladder stones and parasites may have played a role in the formation of tumours. But, on the other hand, the academy report notes that most of the tests which have produced no evidence of carcinogenicity did not involve exposure to saccharin *in utero*. Therefore, they "cannot be interpreted as showing that saccharin is not a bladder tumorigen".

The FDA study and the WARF study were completed early in 1973,



Washington seen

by Colin Norman

but an FDA scientist acknowledged last week that the agency has sponsored no more tests since then, even though doubts have been raised about the role of OTS. The academy therefore recommends a series of studies to investigate the significance of impurities in commercial saccharin and the role of parasites and bladder stones in tumour formation, and epidemiologic studies to see whether there is any relation between cancer and long term consumption of saccharin in man. Dr Julius Coon, of the Thomas Jefferson University in Philadelphia, who was the chairman of the academy committee, said last week that the tests could take about two years to complete.

● No action of Mr Nixon's upset the elders of science in the United States more universally than his decision to shift responsibility for formulating science advice from the White House to the bureaucratic hinterlands of the National Science Foundation. President Ford's announcement last month that he has set Vice-President Rockefeller the task of determining what arrangements should be made for getting science advice to the President was therefore greeted with considerable enthusiasm. But, with Rockefeller occupied in trying to determine the truth of allegations that the CIA indulged in domestic activities, the operation has taken something of a back seat. Henry Diamond, a lawyer connected with Mr Rockefeller's Critical Choices Commission in New York, has been given primary responsibility for the study and some announcement is expected

within the next month or so.

A number of groups, including the National Academy of Sciences and the US Senate, have publicly endorsed the view that a science policy apparatus should be re-established in the White House, and it seems likely that the Rockefeller study will recommend such a move. A key issue, however, will be the extent to which the mechanism will have influence over military research and development—when Nixon scrapped the old arrangement he specifically told the National Science Foundation to keep out of military affairs. And another central factor is how it will interact with the Office of Management and Budget. It is understood that those questions are now being debated among Rockefeller's advisers.

● A Boston physician went on trial last week faced with a manslaughter charge for allegedly killing a foetus during an abortion he performed last year. The trial has drawn considerable attention throughout the United States because it is likely to define in more precise terms when a foetus should be considered capable of surviving independently from its mother.

The physician, Dr Kenneth Edelin, was charged last year by a grand jury of aborting a foetus—described in the charge as a "baby boy"—which was believed to be between 24 and 28 weeks' old, and of failing to take steps to assure its survival.

When the Supreme Court handed down its historic decision on abortion two years ago, it ruled that state laws cannot forbid abortions before the foetus is 'viable', but it deliberately left fuzzy the definition of what constitutes viability. Its decision, in fact, is a model of obfuscation, since it states that viability "is usually placed at about seven months but may occur earlier, even at 24 weeks". The foetus that Edelin aborted is right in that uncertain period, and the trial is therefore likely to hang on the definition of viability.

But, aside from defining more precisely the scope of the Supreme Court's abortion decision, the trial could also have some implications for foetal research. There have, during the past year or so, been several state and national laws enacted which attempt to put a stop to research involving so-called living foetuses, but those laws have largely been passed in the absence of any definition of what constitutes a living foetus. They are generally regarded as greatly restricting some important areas of biomedical research.

The trial is expected to last for four or five weeks, and Edelin could face a lengthy prison term if found guilty.

Moves to set up a register of toxic chemicals

from Peter Collins, Geneva

THE first practical steps towards setting up an international register of potentially toxic chemicals were taken at a meeting at Bilthoven, The Netherlands, this month. Sponsored by the United Nations Environment Programme (UNEP), and hosted by the Netherlands Government, the meeting marks an unexpected change of mood on the part of governments in their approach to a subject which, when raised at the preparatory committee for the Stockholm Conference on the Human Environment, seemed one of those on which early action was least likely. During the committee's discussions the suggestion for such a register had a consistently lukewarm reception, especially from those governments most able to contribute to it. This was largely because of a feeling that so many sensitive areas of industrial confidentiality and, probably, military security were involved that real co-operation on the part of both manufacturers and governments—as users of many candidate materials—would be virtually impossible to achieve.

There has evidently been a big change of attitude, especially on the part of governments, though not yet so visibly on the industrial side. Perhaps the principal reason for this is the sheer pressure under which governments have found themselves when it comes to testing the enormous number of new potentially toxic substances being produced each year, the position of which needs to be defined with regard to the increasingly large and strict body of legislation in many countries, itself in part a result of pressure arising from the atmosphere of the Stockholm Conference.

Governments, it would seem, no longer have the same attitude to confidentiality over much of this field as they did two or three years ago. The more details are made public, the greater is the chance of making widespread testing of potentially dangerous new products more effective, if information is more widely available, testing to acceptable standards can be carried out cooperatively, and the number of candidate substances covered in a given time can be greatly increased. Governments, in fact, are facing a situation in which they cannot, working individually, keep up with their own requirements, since testing to modern standards is so lengthy and costly a process, and a situation already exists

where virtually all the testing is being done in a small group of countries, working informally on behalf of a much larger number of governments. Any facility to help the pooling of information, such as could be provided by an international register, would at least tend to avoid duplication.

As regards industry, it would seem that the gloomy view of the prospects of collaboration at the time of the Stockholm Conference was based on a misconception. The problem is not that industry keeps quiet because it has so much dangerous information to hide. In fact, industry does very little testing beyond a certain point at present, leaving the finer details to governments. What could well be hidden by industry, the UNEP believes, is ignorance of the toxic properties of its own products, rather than information about known dangers. It looks almost as if many companies are keeping quiet because they know so little, not too much.

For these reasons, the proposed register is predicted by the UNEP not on industrial information, but rather on that which is already, and which will be needed, on an international exchange basis. Here again, governments remain the key to the provision of information on which to base any realistic international register.

Some national, and even international, registers do already exist. They vary widely in scope and detail, and help to show up some of the problems that will be involved in any project such as the one the UNEP has in mind. Thus, a list of toxic substances published in the US for the year 1973 runs to nearly 1,000 pages and covers more than 10,000 preparations—none of them described in such detail as the UNEP believes to be necessary for the sort of register it is aiming at. At the other end of the scale come the monographs put out by the International Agency for Cancer Research at Lyons, which now covers more than 100 compounds. These are as complete as could be wished.

In the UK, thinking on how to respond to the UNEP initiative is well advanced. Acting on the advice of the Royal Commission on Environmental Pollution, and of the Royal Society, the Department of the Environment has engaged the UK Chemical Information Service to design and develop a Network on Environmentally Significant Chemicals—DESCNET. This will be so designed as to provide input to, and to draw on, the UNEP International Register. In general, however, the approach in the UK will be selective and will utilise existing systems and data wherever possible.

Meanwhile, the UNEP feels that response to its initiative is widespread. Especially gratifying has been that from

the developing countries, which have a huge problem, inasmuch as they are not, with one or two exceptions, in a position to do any testing for themselves, and have to rely on what information they can gather from published reports or private enquiries elsewhere. The presence of representatives of the Soviet Union and the East European countries, however, perhaps interests the UNEP more at this stage, since there is the hope that from them, at least, information from industry may be available—as it should be, all industry being under government control.

The present meeting differs from previous consultations in this field in another respect: the majority of participants are people actively working on the problems of toxicity or in allied fields, or already concerned in compiling national registers, rather than official government representatives as scientific or administrative civil servants.

Moreover, the common denominator at Bilthoven is the chemical *per se*, not "the chemical in the environment"—in fact, an almost essential approach if the discussion, and the register that is its eventual objective, is to deal with the potential toxicity of new compounds, as well as with the hazards known to attend the use of those already in production. The aim, if the proposed register is to fulfill its purpose, is to satisfy the need for the unambiguous identification of chemicals, and of the dangers that may attend their use, if only because, unless such information is available, it is not possible to computerise the data—and the amount of information will certainly be such that it cannot be handled in any other way. So the intention is not just to list substances that are, or may be, released to the environment, but all sorts of potentially toxic substances, regardless of their categories of use.

Finally, although industry may at present seem to be reacting rather coyly to the idea of such a register, there is little doubt of a basic willingness to collaborate. This is not only because most companies involved in the production of candidate substances are anxious to improve their public image in this respect. It is also evident that while not at present likely to be providers of much information for the register (largely because they do not have the facts required), they will certainly be among the most concerned users of its outputs. The important thing is that we now have the chance of developing an orderly mechanism, internationally and nationally, for assembling an up-to-date dossier on chemical substances as a basis for environmental management and decision making. □

correspondence

Czech persecution

SIR,—I wish to raise some points about Professor Burhop's letter (November 22) Does he know about the existence of a law in Czechoslovakia, which is much more iniquitous than the one which may be accepted in West Germany? This is the Labour Code (law 42/1970), which contains section 46, lett e, according to which a notice could be given to anyone who "violates the socialist social order" In the past five or six years tens (if not hundreds) of thousands of Czechoslovak citizens have lost jobs not only at schools, universities and justice offices, but also in the research institutes, mass media, theatres, publishing houses, museums, state administrative departments and so on because of the application of this paragraph

Having been dismissed myself from the Nuclear Research Institute in 1970 under this paragraph, I tried to get revocation of my notice through the law I shall quote here from the decision of the Court of Appeal, the highest authority in such cases

"The claimant's activity was violating the socialist social order as he signed a resolution which demanded a change in the system of the leading organs (of the Academy) Furthermore, the claimant tried to damage the development of educational and cultural policy of our state, which is led and directed by principles of Marxist-Leninist ideology, and this he did for instance when he pleaded for the abolition of censorship of scientific publications"

Does the World Federation of Scientific Workers raise its voice against this law, which has been in force in Czechoslovakia for many years?

Professor Burhop's version of the shameful persecution of Malek is incorrect Section 11 of the Czechoslovak pension law states that "persons who have reached the age of at least 60 enjoy a right to be retired" No obligatory retirement age is given in the law about the Czechoslovak Academy of Sciences (54/1963)

Professor Malek's contract with the academy had not been extended in October 1973, and Malek had either to find a new job or to retire (in 1970, as a measure of political persecution, the time limited labour contracts had been introduced in the Czechoslovak Academy of Sciences—20, 4, law

26/1970—for senior scientists only The duration of contracts ranges from three months to four years) This dismissal has been an act of purely political persecution or even revenge In contradiction with world academic habits, he does not enjoy the right to keep his office or laboratory in his former institute, he is now not even allowed to use the institute library

I agree completely with Burhop's statement that issues like the retirement age should be "matters for the people of that country to decide" Is he not aware of the fact that the people of my country lost the right to decide on their own fate on August 21, 1968, when our country was invaded by foreign troops which were occupying my homeland?

The plight of Czechoslovak scholars and intellectuals is a direct consequence of this One should not forget it

F JANOUCH

Copenhagen, Denmark

SIR,—I would like to comment on the discussion between Professors Janouch and Burhop (August 9 and September 20) concerning the situation of scientific workers in Czechoslovakia

I absolutely agree with Janouch that "only open, public stands, protests and statements are meaningful and helpful" This is not only based on my own experience while living under this regime but also on experience during work with other institutions, such as Amnesty International There is no doubt that the procedure suggested by Burhop might be effective with some regimes which respect—at least to some extent—reasonably open discussion This is, however, certainly not the case in the so-called people's democracies, where the hypocritical Party bureaucrats not only suppress any sort of criticism but do not even respect their own constitutions and laws

To illustrate the absurdity of the present situation of scientists in Czechoslovakia, I would like to bring up one flagrant example concerning Mr Karel Kriz, a former senior lecturer at the Technical University at Brno (Moravia) Like many of his colleagues, he was dismissed from his position because he declined to accept the Russian occupation as brotherly help For some time he was not able to find any reasonable employment, in 1972, however, he started a "new career" as a buyer dealing in rabbit skins for the national enterprise KARA Since this job was

not very well paid, he was compelled to organise his business using (for Czechoslovakia) new commercial methods he sent various posters and information leaflets (on the eminent importance of rabbit skins for the national economy and so on) to all villages in the southern part of Moravia and informed the inhabitants of his impending arrival In fact, he organised a group of gypsies, who regularly visited all villages and bought up rabbit skins for him and therefore for KARA One of his posters read

Dear friends

KARA purchases hides and skins of every description I shall be calling at your village once a fortnight Kindly have your goods ready for me, Thank you for your cooperation

Your skin buyer

Independent scientific worker

Doc Ing Karel Kriz, Candidate of Sciences, skin buyer for KARA, national enterprise

His action soon became a great success, and the gypsies, villagers and KARA were exceedingly satisfied Even the Party bureaucrats paid tribute to the successful gypsy brigade, which was soon accepted as one of the best "brigades of socialistic labour"

The disaster came in the summer of 1973 in the form of a panel discussion on Austrian Television devoted to the brain drain At the end of the discussion, in which the Prime Minister, Dr Bruno Kreisky, also took part, the commentator said (ironically) that some neighbouring countries had succeeded in solving this problem better than Austria had At the same time he showed Mr Kriz's poster

Two days after the panel discussion, Mr Kriz was arrested on a charge of damaging the interests of the Republic, according to Section 112 of the Penal Code ("A Czechoslovak citizen who damages the interests of the Republic by spreading or enabling to spread in other countries false reports about conditions in the Republic will be punished with prison up to three years") The first trial, which should have taken place in November 1973, was postponed, and in the autumn of 1974 Mr Kriz was sentenced to three years in prison, the maximum punishment What for? After all, the text of the posters was

approved by the censor and according to the law, nothing can prevent Mr Kmz from using his scientific degrees

I would like to end by appealing to all scientists who are lucky enough to live in free democratic countries please, do help actively our colleagues who are deprived of their human rights and are kept in prisons or are dying slowly in concentration camps and psychiatric hospitals, no matter where. If we do not help them, nobody will. After all, passive humanitarianism helps no one except the totalitarian regimes

E. ANTONCIK

University of Aarhus, Denmark

Trieste centre

SIR,—We must indeed be grateful for the letter from Sir David Martin and Mr Cozens (December 20/27) on the policy of the Royal Society UNESCO committee concerning the International Centre for Theoretical Physics (ICTP) at Trieste. Many of us who have been closely connected with the scientific programmes of the ICTP were relieved to learn that the British delegate to the recent UNESCO General Conference praised the centre, and voted for the full proposed financial contribution from UNESCO for 1975 and 1976. To that extent, at least, we have succeeded in our efforts (such as the meeting of the Royal Society on May 16, 1974) to ensure that this committee is fully informed about the work of the centre and is no longer taking decisions without reference to ascertainable opinion amongst British physicists.

The last paragraph of this letter further invites comment on the policy with which the British UNESCO delegation should approach the proposed review of "UNESCO's relations with and subvention to the centre". It is to be hoped that this policy will not be decided without thorough consultation, by the whole committee, with those who understand the situation at Trieste.

A kite is being flown, for example, that the ICTP could be financed mainly by direct contributions from individual governments or national scientific organisations, thus taking the burden from United Nations agencies such as UNESCO and the International Atomic Energy Agency (IAEA). This would be a disaster. As I thought was obvious from my article in *Nature* on March 22, the administrative resources of the ICTP are quite inadequate to the solicitation and collection of such contributions. It is able to carry out a valuable scientific programme on behalf of all nations, without serious political disruption, because it is a technical instrument through which a small number of international agencies (including the UN Development Program and the Swedish International Development Agency) channel their

efforts. Additional contributions from individual governmental organisations, such as Britain's Science Research Council, are very welcome, and are not inappropriate considering the scientific work that is actually done at Trieste by physicists from Britain and other advanced countries, but there is no substitute for the basic budget, shared by UNESCO, the IAEA and the Italian Government, that keeps the centre in active being. The real questions, rather, are the formal place of the contribution to the ICTP in the UNESCO budget, and the administrative devices by which it is maintained as a permanent institution within both UNESCO and the IAEA.

In view of the time and trouble it has taken in the past to get the facts about Trieste across to the British UNESCO delegation, it is not too early to air these issues in your columns (or in some suitably ventilated corridor!) in preparation for a wise decision in 1975 or 1976.

JOHN ZIMAN

University of Bristol

Hungarian attitudes

SIR,—Dr Peller's Hungarian experience (December 13) will only surprise those who believe that Hungary is a 'liberal' east-block state. In a very restricted sense this is true, but life for research workers in Hungary is difficult, and especially so if they intend to travel westwards. Outward evidence of the internal situation is shown by the difficulties Western scientists sometimes encounter if they want to travel in Hungary. As a former coworker in a research institute in Budapest, I had the opportunity of participating in the Conference of Solid State Physics held in Manchester in January 1966. I also intended to make a contribution and, as is the usual case in Hungary, I should have received by passport just one day before my travel. On that very day the Ministry for Metallurgical and Engineering Industry, my 'higher' authority, told me that I would not get a passport. No reason for this decision was given. This was only one typical case among several similar ones during the 17 years of my scientific career in Hungary. In general, for a young scientist, professional travels to Western countries are possible only if he is 'politically reliable'.

Undoubtedly only few entry applications are rejected. Israelis are especially not welcome, probably because of the great number of Jews living in Budapest, who might get authentic information on the real situation in the Middle East. Western scientists who have participated in a conference in Hungary, however, are in general delighted by the traditional hospitality of the organisers and this

helps to mask the autocratic treatment of the international scientific communities by the authorities.

LAJOS ERNST

West Berlin

University consultancy

SIR,—I would like to support Mr H. A. Cook's letter (December 13). Although I am not certain that all consultancy by university staff should be discouraged, for after all this is one way in which the results of research are transferred to industry, I am more particularly concerned at the use of university facilities for the production of hardware for commercial purposes.

In this company we are aware of at least two instances in which companies have been formed by university staff to manufacture equipment which is in direct competition with some of our own products. We are also aware that these companies have no manufacturing facilities of their own and that university plant, equipment and man-hours are used in the production of this equipment.

In effect, such companies have extremely small overheads which makes competition very difficult for a company like ours where all the research and development work must be paid for from profits.

I agree completely with Mr Cook that the appropriate government department should put a stop to the extracurricular activities of those university staff who, in effect, are being paid two salaries for doing one job and are using public funds under false pretences.

J. B. S. PRICE

Grubb Parsons,

Newcastle upon Tyne, UK

Periodic unification

SIR,—In your issue of October 25, page 661, paragraph three, there appeared an anonymous quotation and comment about the reception of some of Mr Edward Haskell's work, referring to a conference held in New York in 1971. The item "'We had to listen to some extraordinary ideas of unification by way of Mendeleev's periodic table' said one participant. Another was horrified that whilst the scientists thought it silly, the philosophers present took it very seriously." I wish to set the record straight. There may have been more than one scientist who thought Haskell's work silly—blindness that was found in attitudes of some scientists towards precursors of Mendeleev's table. I can, however, certify that not only the philosophers present but also a number of highly qualified scientists who were also there, and who read Haskell's work, take it very seriously indeed.

HAROLD G. CASSIDY

Texas Christian University, Fort Worth

news and views

Protein turnover in the whole body

from J C Waterlow

In this issue of *Nature* (page 192) Young and co-workers at the Massachusetts Institute of Technology report measurements of the rate of protein synthesis in the whole body in human subjects from birth to old age. This is a timely contribution to a topic which is attracting increasing attention, and which has lain fallow for some time. It is 35 years since Schoenheimer, Ratner and Rittenberg (*J biol Chem*, **127**, 333, 1939) reported "the first experiment in which one amino acid of a normal diet, tyrosine, has been labelled by the nitrogen isotope". In subsequent papers the concept of protein turnover was firmly established, but it was not for another 10 years that attempts were made by two groups, that of Rittenberg at Columbia and of Wu in Alabama, to measure the overall rate of this process in the whole body (Sprinson and Rittenberg, *J biol Chem*, **180**, 715, 1949; Wu and Snyderman, *J gen Physiol*, **34**, 339, 1950). In these early studies the method used was to give a single dose of an amino acid labelled with ^{15}N and to calculate the rate of turnover from the curve of ^{15}N excretion over the next 48 hours or so. It was soon realised that such a calculation is erroneous: the shape of the early part of the excretion curve is the resultant of a large number of processes, and cannot be analysed in a meaningful way. After the first few hours the slope is determined largely by the turnover rate of urea and not by that of protein.

From this point development occurred in two directions. Olesen *et al* (Olesen, Heilskor and Schønheyder, *Biochim biophys Acta*, **15**, 95, 1954) measured ^{15}N excretion in urine for 15 days after a single dose of ^{15}N -glycine, and calculated the rate of protein synthesis by multi-compartmental analysis. Theoretically this method is an improvement on the earlier ones, in practice it has not been followed up, presumably because of the difficulty of maintaining a constant nitrogen intake and a steady state in subjects other than healthy volunteers. San Pietro and Rittenberg (*J biol Chem*, **201**, 457, 1953) took an opposite line. They devised a method which depends on measuring the maximum ^{15}N abundance in urinary urea after a single dose of ^{15}N . Peak labelling occurs about 3 hours after the dose, and the calculation of

protein turnover depends upon this interval being accurately timed. In fact it is impossible, from measurements on urine, to know precisely the time at which maximally labelled urea was synthesised. The method is therefore unsound. In spite of this it was quite widely adopted in subsequent years to study protein turnover in various pathological states such as pituitary and thyroid disease. But the results in normal subjects showed a wide scatter, and nothing of interest was revealed in patients with metabolic disease. Probably for this reason interest in the subject waned. It is unfortunate that the method most widely used was the least reliable.

Nothing more seems to have been attempted for about 10 years until Picou and Taylor-Roberts (*Clin Sci*, **36**, 283, 1969) working on malnourished infants in Jamaica, used a continuous infusion of ^{15}N -glycine to produce an isotopic steady state. If nitrogen balance is measured as well, total rates of both synthesis and breakdown can be calculated. This is the method which six years later has been used to good effect by the workers at MIT. It probably gives more accurate estimates of total protein turnover than the older methods, but problems remain. On theoretical grounds a likely source of error is that even in the steady state the labelling of amino acids in the precursor pool for urea synthesis may not be the same as that of amino acids for protein synthesis. The report of Young *et al* suggests however that the method gives results which at least have comparative meaning. Moreover, the value for protein turnover in normal adults, of $3 \text{ g kg}^{-1} \text{ d}^{-1}$ agrees well with results obtained by infusion of radioactive amino acids, whereby the special problems associated with urea formation are avoided (James, Sender, Garlick and Waterlow, unpublished).

It seems reasonable to suppose that

the difference in turnover rate in infants and adults is related at least in part to growth. A major contributor to whole body turnover is the turnover rate of muscle protein. Table 1, from unpublished results of D J Millward, shows the rates of synthesis and breakdown of muscle protein in rats of various ages. The difference represents net growth of muscle protein. Rapid rates of growth are accompanied by rapid rates of turnover. From the energetic point of view this is a costly process. The energy cost of protein synthesis is not negligible, estimates range from about 4 kJ per gram, based on the requirement of 4 moles ATP+GTP per mole peptide bond, to about 30 kJ per gram, derived from nutritional measurements. Whatever the true figure *in vivo*, it looks as if protein turnover must account for a considerable part of the basal energy expenditure. A general relationship was suggested between rates of protein turnover and of basal metabolism (Waterlow, *Lancet*, **ii**, 1091, 1968) and Young's work confirms this.

There remains a substantial gap between rates of protein synthesis at the subcellular level and in the whole animal. The information that is becoming available about synthesis rates *in vivo* shows that the efficiency of synthesis (g protein synthesised per g ribosomal RNA) in subcellular systems is perhaps 100 times less than the efficiency in the whole animal (Henshaw *et al*, *J biol Chem*, **246**, 436, 1971; Millward *et al*, *Nature*, **241**, 204, 1973). Until this difference is reduced, we can rely on subcellular systems for information about mechanisms but not about rates. A major problem for the future is to understand the means by which rates of protein turnover are controlled. It is perhaps provocative to suggest that, in spite of all the difficulties, a solution is most likely to come from studies on the whole animal.

Table 1 Rates of muscle protein synthesis, breakdown and net growth in normal hooded rats at different ages

Age (d)	Fractional rate (% d ⁻¹)			Efficiency of synthesis (g protein/g RNA/d)
	Synthesis	Breakdown	Net growth	
23	28.6	22.4	6.2	19.2
46	16.1	13.1	3.0	14.2
65	11.5	9.8	1.7	13.8
130	5.3	4.6	0.7	10.1
330	4.9	4.6	0.3	11.5

Antibody structure and antigen binding

from C C F Blake

ONE of the major problems in immunology has been the reconciliation of the structure of antibodies with the vast range of antigen binding properties they possess. The long and patient study of antibody structure by chemical methods has produced a basic framework for the solution of the problem by showing that antibody molecules have the following features: (1) the molecules are built from two identical heavy chains of ~440 residues and two light chains of ~220 residues, linked together by disulphide bridges, (2) there are four repeating homology regions or domains of ~110 residues in the heavy chains, and two in the light chains, (3) the amino-acid sequence of the N-terminal domains is variable, (4) the sequence in the rest of the molecule is more constant and allows the chains to be characterised as κ and λ light chains which can associate randomly with α , γ and μ heavy chains to give respectively the IgA, IgG and IgM major isotypic classes of antibody molecules, (5) within the variable sequence there are hypervariable regions. This information has been seen to imply that antibody molecules have a common basic structure, in which the antigen binding function is carried by the variable domain whose hypervariable regions are involved in the binding process.

For several years protein crystallographers have been seeking to give this elegant and satisfying model its final structural definition, and at the same time to provide a molecular description of the binding site and its mode of interaction with antigen. As with other studies in this field the work has been hindered by the heterogeneous nature of antibodies and to some extent by the size of the molecule. The former problem has been overcome in the usual way by using myeloma proteins, and the latter, although preliminary X-ray studies on whole immunoglobulins have been reported, by utilising fragments of the molecule, either the papain-induced Fab fragment which contains the binding site, or the naturally occurring Bence-Jones protein, a dimer of the light chains.

In the last year or so, four groups have independently reported high resolution structural analyses of these fragments. Poljak and his colleagues have worked on the Fab fragment of human IgG1 New (Poljak *et al*, *Proc natn Acad Sci USA*, **70**, 3305, 1973; Poljak *et al*, *ibid*, **71**, 3440, 1974), Davies and his group on a similar fragment of mouse IgA, McPC 603 (Padlan *et al*, *Nature new Biol*, **245**, 165, 1973; Segal *et al*, *Proc natn Acad Sci, USA*, **71**, 4298, 1974) and the groups led by Edmundson (Schiffer *et al*, *Biochemistry*, **12**, 4620, 1973) and Huber (Epp *et al*, *Eur J Biochem*, **45**, 513, 1974) have each solved

a human Bence-Jones protein. In confirmation of the chemical model all four groups report essentially similar tertiary and quaternary structures.

In the X-ray models the homology domains of ~110 residues appear like beads threaded on a string. Each domain has a tertiary structure that is basically two parallel β sheets between which is the hydrophobic core. The intrachain disulphide bridge, which is a characteristic of each domain, links strands in opposite sheets so that the two sulphurs are located near the centre of the domains. In the constant domain one sheet has four strands and the other three, the variable domain has an additional loop that extends the three-stranded β sheet of the constant domain to five strands. The link between the two domains is made by a short extended chain segment called the 'switch' region.

Each domain associates non-covalently with an equivalent domain in the other chain: the Fab has $C_{H1}C_L$ and V_HV_L dimers, and the Bence-Jones C_LC_L and V_LV_L dimers. This domain dimerisation has two unexpected features. The constant domains associate so that the four-chain sheets are closely opposed and their strands approximately perpendicular, but the variable domains oppose the other β sheet (the one with five strands) which leads to a looser association and a greater intersheet spacing. In the association each domain is related to the other in the pair by pseudo two-fold symmetry, but all four groups have found that the axes of symmetry between the pairs of constant and variable domains are far from colinear, angles of intersection between 120° and 135° are reported. As a result the switch regions bend through different angles in the two chains to give the four domains a tetrahedral arrangement. Although this is not altogether surprising in the Fab where the four domains are chemically distinct, the lack of structural equivalence between the chemically identical chains of the Bence-Jones proteins has led Schiffer *et al* to suggest that one of the two light chains in the molecule fulfils the structural role of the heavy chain in Fab. This suggests that Bence-Jones proteins are counterparts of the antigen binding regions of immunoglobulins and may themselves have functional binding sites.

The close similarity of tertiary and quaternary structure observed in the Fab fragments and the Bence-Jones proteins strongly implies the invariance of many features in the architecture of antibody molecules, and allows some important predictions to be made. The amino acid homology between the C_{H2} and C_{H3} domains of the Fc fragment and the C_{H1} domain of Fab makes it

highly probable that the X-ray analysis of Fc now under way will reveal an equivalent structural homology. Poljak has shown from his results that the intrachain disulphide bridge in human γ_2 , γ_3 , γ_2 and μ chains, which is shifted by 83 residues on the heavy chain from its position in the γ_1 heavy chain in his molecule, can be made without altering the observed folding of the polypeptide chains. He also shows that a number of additional or unusual disulphide bridges which have been observed are made between residues that are already in the appropriate orientation. This is additional strong evidence that the results we already have in the Fab parts of the molecule provide the structural basis for all other classes of antibody molecule.

The properties of the antigen binding sites of the two Fab fragments have been investigated by binding their appropriate haptens: vitamin K to Fab New (Amzel *et al*, *Proc natn Acad Sci USA*, **71**, 1427, 1974) and phosphorylcholine to Fab McPC 603 (Segal *et al*, *ibid*, **71**, 4298, 1974). In addition Edmundson, following his suggestion of the analogy of Bence-Jones proteins to Fab, has examined the binding of dinitrophenyl ligands to his protein (Edmundson *et al*, *Biochemistry*, **13**, 3816, 1974). In all cases binding takes place at a cavity between the variable domains, but the cavity has a different size and shape in the different molecules. Amzel *et al* describe their binding site as a shallow groove 15 Å × 6 Å × 6 Å while Segal *et al* find a large wedge-shaped cavity 12 Å × 15 Å × 20 Å and Edmundson a conical cavity 10 Å in diameter leading to a deep pocket. The groove or cavity is lined mainly or exclusively with residues from the hypervariable regions of the sequence, which in the structure cluster to form the surface of the binding pocket. Segal *et al* argue from their results that amino acid substitutions, deletions and additions in these regions could induce such differences in the overall shape, size and general chemical nature of the antigen binding sites that the hypervariability itself could be sufficient structural explanation for antibody diversity. On the other hand neither group working on the Fab fragments has observed any significant conformational change on hapten binding of the kind that might be expected to provide a structural basis for complement fixation or B-cell activation reactions (both functions of the Fc fragment). Nevertheless, Poljak *et al* do not rule out such a possibility and point to the asymmetric orientations of the switch regions as possible loci of structural alteration, which perhaps takes place when the true antigenic determinant rather than a simple hapten is bound.

THE continued existence of the apparently unstable West Antarctic Ice Sheet and Ross Ice Shelf is a major geophysical puzzle that only recently has been recognised. In this issue of *Nature* (page 168) Dr Gordon Robin presents new field data obtained on the Ross Ice Shelf and the very fast moving ice streams that drain the West Antarctic Ice Sheet. These field data are essential to the future solution of the puzzle.

The West Antarctic Ice Sheet forms one continuous body of ice with the Ross Ice Shelf. By definition the ice sheet becomes the ice shelf where the ice loses contact with bedrock and floats in sea water. The base of the West Antarctic Ice Sheet over much of its area is at the startling depth of 0.5 to 1 km below sea level. More over the bed would remain below sea level over a large area even if all the ice were removed and isostatic rebound took place. Only the great weight of the ice sheet keeps the ice pressed against bedrock. But at the edge of the ice sheet the ice thickness is insufficient to keep the ice mass grounded and here the floating ice shelf is formed. The ice thickness at the junction of the ice sheet and the ice shelf is greater than that of an equilibrium ice shelf that is nourished only by the snow that falls on it. Thus the Ross Ice Shelf becomes thinner the nearer it is to its seaward edge.

Stability of Antarctic ice

from J Weertman

It is natural to suspect that an ice sheet whose base is so far below sea level might be unstable. Hughes (*J geophys Res*, **78**, 7884, 1973) has suggested that the West Antarctic Ice Sheet is disintegrating. He thinks the junction of this ice sheet with the Ross Ice Shelf is retreating at present at a rate of about 70 m yr^{-1} . This rate of destruction of the ice sheet would raise the sea level by about 0.5 mm yr^{-1} .

On the basis of a simple-minded, two dimensional glacier mechanics analysis I found, essentially, that there is a 50-50 chance that the West Antarctic Ice Sheet is indeed disintegrating (*J Glaciol*, **13**, 3, 1974). A more accurate answer to the question of whether it is disintegrating and if so, how fast should be obtainable from a three dimensional glacier mechanics analysis carried out with the aid of computer calculations. Such an analysis requires the input of extensive field data from the West Antarctic Ice Sheet that have yet to be gathered as well as the field data that Robin has obtained for the Ross Ice Shelf. The question can, of course, be

answered with field observations. But it may be very difficult to determine where the Ross Ice Shelf joins the West Antarctic Ice Sheet if, as seems to be the case, there is no sharp line of demarcation. There seems to be a broad zone over which the ice alternately floats and is grounded. Furthermore, if the rate of retreat is of the order of Hughes's estimate, field measurements made over many decades would be required to obtain a reliable answer.

Robin's data on the ice streams may help solve another problem that could be related to the stability of the West Antarctic Ice Sheet and to which not even a tentative solution has been proposed in the literature: namely, why fast moving ice streams form near the edge of this ice sheet. Almost all of the ice that flows from the West Antarctic Ice Sheet moves through such streams which could be formed either in deep subglacial channels or because of an instability in the ice flow. There seems in fact to be no good field evidence that the ice streams are confined in deep channels in the bedrock. If the streams do not correspond to channels in bedrock their widths will be unstable and the West Antarctic Ice Sheet might surge by a rapid increase in the width of an ice stream. One theory of the ice ages is based on postulated surges of the Antarctic Ice Sheet.

Structure of a structural protein

from D L D Caspar

PROTEINS which build viruses, muscle, membranes and other cellular assemblies stand in a separate class from the soluble proteins of protoplasm and extracellular fluids. D A Marvin and his colleagues at Yale have now reported the construction of the first realistic molecular model of an assembly of a structural protein. The structure is a slender bacterial virus called Pf 1 which now becomes a robust paradigm for essential macromolecular assemblies in cells and tissue.

Marvin's quest for the structure of the filamentous bacterial viruses started a dozen years ago when these μm long, 60 Å diameter particles seemed a simple model system for defining the minimal properties of a self-assembling virus. His first X-ray patterns from fibres of the fd strain showed key features characteristic of α -helical fibrous proteins: strong diffraction near the equator at ~ 10 Å spacing and near the meridian on a ~ 5 Å layer line. Spectroscopic measurements indicated that the coat protein might be all α -helical in the intact particle, and the sequence of

the 50 residue fd subunit suggested which end would interact with the DNA core. Possible α -helical models for the subunit were built long before there was any clear idea of how to pack them in the virus particle. The problem was that the symmetry of the subunit packing was hidden in the X-ray pattern. Four other strains of this virus isolated in North America and Europe gave similarly inscrutable X-ray patterns. Attempts to figure out the coat protein design from misleading electron micrographs led to fanciful two-stranded models. Marvin, Wiseman and Wachtel (*J molec Biol*, **82**, 121, 1974) solved the packing problem by looking at the structure of two more filamentous viruses: Pf 1 isolated in Japan and Xf from China. These oriental strains gave readily scrutable X-ray patterns since they have regular helical symmetry (4.4 units per turn of 15 Å pitch). Moreover, the occidental strains could be seen by comparison to have similar underlying symmetry which was obscured by quasi-equivalent bonding of the subunits in a periodically

perturbed helix.

Marvin and Wachtel (*Nature*, **253**, 19, 1975) have gone on to solve the structure of the α -helical subunit in the oriental Pf 1 strain by first tracing out its backbone. This is defined by the near equatorial diffraction in the 10 Å region of the X-ray fibre diagram and by the rules for packing α helices in coiled coils which had been formulated by Crick in 1953. The α -helix axis is coiled into a segment of a left-handed conchospiral. This is the symmetrical curve on a conical surface which describes the form of conch shells and other growing helices. Each unit in the spirally thatched virus sheath interlocks over much of its length with the fifth and ninth units along the helix of 15 Å pitch. The side-chain packing cannot be seen from the X-ray pattern alone. Marvin's approach to this problem has been to fit the amino acid sequence of Pf 1 determined in collaboration with Nakashima, Wiseman and Konigsberg (*Nature*, **253**, 68, 1975) to the constraints set by the X-ray diagram and the stereochemistry. The acidic N-

terminus of the 46 residue molecule is placed outside and the basic groups near the C-terminus are arranged inside to neutralise the nucleic acid core. Following criteria worked out by Smillie and his colleagues (Hodges *et al*, *Cold Spring Harb Symp quant Biol*, **37**, 299, 1972) for "knobs-into-holes" interlocking of the tropomyosin side chains in a coiled-coil, the Pf 1 subunit can be arranged so that most of the hydrophobic residues make favourable van der Waals contacts with those on neighbouring α helices. It looks as if this ingenious model building from sequence data refined against a low-resolution X-ray fibre pattern may lead to as clear a picture of the atomic structure of this virus as protein crystallographers now produce by automated analysis of high resolution diffraction data from isomorphous crystals.

Structural molecular biology started with model building. Pauling and Corey discovered the α helix in 1951 by folding a stereochemically correct polypeptide chain to optimise the interactions between turns. Crick looked at the interactions between chains and constructed his models for α -helical coiled coils as well as his half of the double helix in 1953. Models of these helical molecules could be built and refined since the backbone folding was seen directly in the X-ray fibre diagrams. The chain folding in globular proteins is not, however, apparent from inspection of the forest of spots in single crystal diffraction patterns. Another breakthrough in 1953 was Perutz's introduction of the isomorphous replacement method which led the way for development of protein crystallography from what he called (in a different context) "the ingenious puzzle-solving of the early pioneers to the almost blindfold automation of the present day". The structures of many soluble globular proteins which yield excellent crystals have now been solved to atomic resolution by this technique. Crystallographic scrutiny has not been comparably successful with structural proteins since good crystals with small unit cells are uncommon—perhaps because these proteins are designed to aggregate in their own way. Isometric virus particles, enzyme complexes and other globular protein assemblies have been crystallised and these structures may be solved by crystallographic methods in spite of the very large unit cells. Structural proteins with recognisable backbones—such as tropomyosin filaments which give relatively poor crystals—will be solved like the filamentous phage by refining a molecular model against low resolution X-ray data.

The structure of a virus or a cellular assembly embodies the rules for its formation. The filamentous bacterio-

Visions of a laser accelerator

from O S Heavens

A LITTLE over twenty years ago, Smith and Purcell observed the emission of light from a metallised diffraction grating when a 300 keV beam of electrons was directed along the surface in a direction perpendicular to the rulings. This phenomenon, which is referred to as the Smith-Purcell effect, is easily understood in terms of the oscillations induced in the charge carriers in the grating consequent upon the passage of electrons close to the surface. Oscillations occur, minute dipoles are created, and for the appropriate choice of electron velocity the radiation emitted will lie in the visible spectrum. The effect has attracted a modest interest and several papers have appeared giving detailed theoretical accounts of the effect, which can be understood conveniently in terms of leaky space-charge waves from periodic structure, generally considered as alternately conducting and insulating strips.

Curiously enough, the emphasis has been entirely on the radiation produced by the interaction of the electron beam with the structure. The reverse effect—in which we ask what would happen to an electron beam near a periodic structure when an electromagnetic wave passes over the grating—seems not to have been considered until recently (Mizuno, Ono and Shimoe this issue of *Nature*, page 184). The intriguing possibility arises of using a coherent (laser) beam in such a way as to produce an accelerated beam of electrons. Accelerations of the order 1 GeV per metre seem to be feasible, coupled with the possibility of current pulses as short as 10^{-15} second, thus opening a new time domain to the experimentalist. It may be early to speculate that the typical large, expensive particle accelerator will become a white elephant, displaced by a laser of relatively trifling cost and an old diffraction grating. If this happens, and if laser fusion becomes a reality, optics may cease to be dismissed as an unfashionable old-hat topic neatly finished by one J C Maxwell!

phage is not constructed by simple self-assembly of coat protein and nucleic acid even though the design of the particle is determined by the specific bonding properties of its parts. A regulatory virus protein combines with the

viral DNA strand in the cytoplasm thereby segregating it from the replicating circular duplex and controlling its synthesis as a separate circle. The coat protein does not appear in a soluble form in the cytoplasm but is incorporated as it is made into the plasma membrane of the bacterium. Particle assembly proceeds by extrusion of the pre-packaged circular DNA molecule through the membrane bilayer where coat protein displaces regulatory protein. The assembly process is reversed in infection. Following attachment to a susceptible host by the A-protein located at one end of the virus filament the coat protein dissolves in the membrane bilayer as the DNA and A-protein are transported into the bacterium.

In the bacterial membrane, the virus coat protein may still be folded in its α -helical conformation stabilised by the oily environment, and it may span the bilayer with its positive and negative ends on opposite sides. This picture is reminiscent of many speculative models for membrane proteins—and one recent experimental one. Richard Henderson at the MRC laboratory in Cambridge has recently seen by X-ray diffraction that the purple membrane protein of *Halobacterium halobium* is folded into α -helical segments directed approximately normal to the plane of the bilayer and these segments appear to be packed together with coiled-coil interlocking (*J molec Biol*, in the press).

The filamentous bacteriophage in its different stages of development may be as useful for understanding membrane differentiation as it will be for comprehending fibrous protein organisation, regulation of DNA synthesis and control of virus assembly. Marvin has revealed this structure as a microcosm in which these basic problems in structural biology can be critically explored.

Molecular weight distribution in polymers

from Paul Calvert

GEL permeation chromatography (GPC) is the only satisfactory method of studying molecular weight distribution in synthetic polymers. Recent improvements in column packing materials and in molecular weight sensitive detectors should allow the development of this technique from a slow, poorly calibrated measurement into a rapid accurate one.

Synthetic polymers owe their utility to their high molecular weight, and average molecular weight is a much measured characteristic. But some important

properties are particularly sensitive to the molecular weight distribution as well as to the average for example a small fraction of very long molecules will markedly increase melt viscosity and a few short molecules can disproportionately reduce the breaking strength

Separation in GPC is due to differential exclusion of the randomly coiled macromolecules from pores in the particles of the column packing. Larger molecules are excluded from more pores and flow faster through the column. In operation the technique is clumsy. High temperatures are needed to keep many polymers in solution and this temperature must be closely controlled along 3–4 foot columns. High pumping pressures are needed but pressure variations must be minimised. The column packing may be cross-linked porous polystyrene particles, which are difficult to pack and use, or porous glass particles, but adsorption effects make such glass packings unsuitable for polar polymers. The eluting polymer is usually detected by differential refractive index measurement. A typical run takes 2–3 hours and a change of the solvent system is a major operation. Ideally, calibration would be performed using narrow molecular weight distribution polymers under the same conditions as the subsequent runs, but only for polystyrene are such fractions readily available. This problem is avoided by use of a universal calibration curve based on the assumption of separation by molecular volume and on known viscosity-molecular weight relationships for the polymer under run conditions. This calibration has often been questioned—for instance Otsuka and Hellman in *J Polymer Sci* (letters) (12, 331, 1974), show that it is successful for polystyrene on glass bead columns in a range of solvents but not on polystyrene-bead columns where the pore size is probably also a function of solvent.

Thus GPC is at present suitable for application where a large number of similar samples are to be measured but lacks the flexibility or accuracy necessary for research applications and is too slow for on-line process control.

Two papers by Ouano and Ouano and Kaye describe progress in the development of a molecular weight, continuous flow detector which would eliminate the calibration problem. The first (*J Polymer Sci* (A-1), 10, 2169, 1972) described the construction of a viscosity detector which measures the pressure drop in a capillary. Molecular weight could be determined to within 10% in a 10 μ l cell at concentrations of less than 10^{-1} g l $^{-1}$ and flow rates of 1 ml min $^{-1}$. The main drawback is the sensitivity to pump pulses.

A recent paper (*J Polymer Sci*

(Chemistry), 12, 1151, 1974) describes a low angle laser light scattering detector using a 6 μ l cell which will work at concentrations of 10^{-2} g l $^{-1}$, for molecular weights from 10^2 to 10^6 . Between the column and the cell a filter is necessary to remove fragments of column packing. The volume of the filter and its connecting tubing significantly reduces the resolution of the present system.

High speed GPC is a second recent development which is being studied in several laboratories. Unger *et al* (*J Chromat*, 99, 435, 1974) describe a system using polystyrene gel particles in which a 2 foot long column of 5 μ m particles could separate a polystyrene sample in 10 min, at a flow rate of 2.5 ml min $^{-1}$. Ultimately high speed must be at the cost of resolution but the use of small particles offsets this.

Radioactive tracers in the atmosphere

from D. H. Pearson

THE use of fission product pairs with similar half-lives as meteorological tracers is described in a recent paper (Holloway *et al*, *Journal of Geophysical Research*, 79, 4453, 1974). This is a recent example of the exploitation of radioactive tracers in the atmosphere, a technique that has developed considerably during the last twenty years.

The impetus came during the 1950s when it became necessary to determine the distribution of nuclear weapon debris by taking samples of airborne dust or rainwater and to attempt a quantitative estimate of the radioactivity in order to assess the radiological hazard. At first it was possible only to measure the gross radioactivity of the sample and then make corrections for decay, according to a crude assumption that the beta-radioactivity of the fission product mixture varied more or less inversely with time. Soon however the full sensitivity and resolution of the radioactive tracer method became available when samples of airborne dust and rainwater could be analysed for specific radionuclides.

The earliest and probably most rewarding achievement of this technique was the confirmation of the seasonal reinforcement of the troposphere in the early summer of each year by air from the stratosphere. The variation of the concentration of strontium-90 (half-life 28 year) in rainwater, and subsequently caesium-137 (30 year) in air, gave ample demonstration of the effect which had been previously indicated by the behaviour of ozone and water vapour in the atmosphere. At the same time it was possible to show that the radioactivity transferred from the stratosphere appeared in the troposphere with a maximum in mid-latitudes and minima at pole and equator.

The next advance in exploitation of the technique was to extract qualitative as well as quantitative information from these observations by measuring the radioactivity of

pairs of fission products. Thus the ratio of strontium-89 (50 day) to strontium-90, of cerium-144 (285 day) to caesium-137 and of various other combinations could be used to estimate the date of the fission explosion, or if the date was known then these ratios could indicate the proportion of nuclear debris released by the latest explosion against a background from earlier events.

Because the bulk of fission radioactivity has been inserted into the stratosphere it is possible to determine, by observations of these radioactive tracers, the delay of stratospheric residence time before the material returned to the surface of the Earth. The massive explosion series of 1961–62 were followed by several quiet years when it was possible to determine unambiguously a mean residence time in the (lower) stratosphere of about 16 months by measuring the decrement of the long-lived fission products strontium-90 and caesium-137. It was also possible to show, during this period when the northern hemisphere contained so much more fission radioactivity than the southern, that the mean residence time against interhemispheric transfer was between three and five years.

Meanwhile the radioactive tracer method has been used with natural radioactivity, derived both from the surface of the Earth and from cosmic radiation. Radon-222, emanating from terrestrial radium, has a succession of radioactive descendants, the longest-lived of which are lead-210 (22 year) and polonium-210 (140 day). These two tracers have been used, separately or relatively, as an index of the residence time in the troposphere, the resuspension of dust and the prevalence of continental or oceanic winds. A number of radioactive species are produced by the interaction of cosmic radiation with atmospheric nuclei. Of these, for example, the ratio of beryllium-7 (54 day) to phosphorus-32 (14 day) has been used to indicate vertical movements in the atmosphere.

These run times make possible process control using GPC

The availability of rapid, accurate GPC would be a great boost to polymer research, in studies of both polymerisation and polymer properties. Many effects which are attributed to changes in average molecular weight may turn out to be more directly related to changes in the distribution. In principle, these techniques would also extend the ability of porous gel (Sephadex) columns to separate complete mixtures of proteins, it will be interesting to see if they are taken up

Aerosol and climate: hotter or cooler?

by John Gribbin

THE puzzle of whether an increase in dust in the atmosphere leads to global cooling or global warming is given a further airing in a recent issue of *Science* (186, 827, 1974). According to some authorities this is a problem of extreme urgency, but the one sure conclusion from the latest results is that it cannot be solved by simple approximations

Just such a naive estimate led to the original fears that man-made aerosols (that is, small dust particles, not the gases used as propellants in so-called 'aerosol' sprays) might be blocking out enough of the Sun's heat to produce a significant cooling of the Earth, perhaps leading to a new ice age. Volcanic dust had already been suggested to contribute to the onset of natural ice ages, and experiencing a hazy day will suggest to most people that dust in the air reduces the temperature. Although such dust must prevent some of the incident radiation reaching the surface, however, the question remains of how much radiation is absorbed by the dust, and to what extent the dust prevents radiation from the Earth's surface escaping into space. These are non-trivial points, as calculations by Weare, Temkin and Snell emphasise. In simple terms, the problem

becomes one of whether the 'grey' particles of dust are distributed above a strongly reflecting 'white' ground surface, or above an absorbing 'black' surface. Earlier in 1974 Chýlek and Coakley reported calculations in which an analytical solution of the radiative transfer equation produces a guide to whether aerosol particles will lead to heating or cooling for any given albedo of the underlying surface. The critical ratio is that of the absorption cross section $(1-\omega)$ to the average back-scattering cross section $(\omega\beta)$ for the aerosol particles. When this exceeds a certain critical value, heating occurs, when it is less than that value there is cooling. And this critical value is related to the albedo of the underlying system (a) by $(1-\omega)/\omega\beta = (1-a)^2/2a$, where ω is the particle single scattering albedo (*Science*, 183, 75, 1974). This confirms that the same type of aerosol will have different effects over surfaces with different albedos and seemed to provide a guide to how these effects would vary. But the further study by Weare *et al* now shows the importance of at least one effect neglected in the earlier calculations.

This is the question of just where in the atmosphere the aerosol layer produces its greatest effect. The relation between aerosol properties and underlying albedo found by Chýlek and Coakley is restricted to the situation when the aerosol layer is distributed above the Earth-atmosphere system—and that is clearly a less than realistic situation. Numerical calculations for the cases when the aerosol layer is above or below the cloud layer, and for a "well mixed" model, show, not surprisingly perhaps, that "at any given preexisting Earth-atmosphere albedo, in order for added aerosol to result in heating, the critical absorption-to-backscatter ratio for the cloud is least when the aerosol is distributed above the cloud." For an annual global average albedo of 0.29, the critical ratio is 0.9 for an aerosol layer above the cloud, 1.3 in the well mixed model, and 3.2 for an aerosol layer between

the cloud and the Earth's surface

But even these calculations contain sweeping generalisations which require that they be used only with extreme caution in the real world. The "global average" approximation is itself an imperfect guide to reality, the calculations assume diffuse thin cloud rather than a 'lumpy' distribution of cloud cover, and aerosol particles from man's activities do not, by any means, cover the entire globe. The important conclusion from this work is that the effect of added aerosol on the radiation balance of the Earth does indeed depend both on the intrinsic optical properties of the aerosol particles and on their distribution through the atmosphere, as well as on the reflective properties of the underlying surface.

The situation also has an analogy in the problem of how aerosol particles might affect cloud cover. Acting as 'seeds' for droplet formation, they might increase cloud cover, increase the Earth's reflectivity, and produce a global cooling. On the other hand, if the particles simply attach themselves to existing droplets then 'dirty' clouds are produced, which could absorb more radiation and produce a global warming. It seems foolish for mankind to tamper with such a complex and ill understood system, and careless pollution of the atmosphere is unlikely to be a good thing. But we are still far from being able to say with confidence whether or not these polluting activities are hurrying the onset of the coming ice age.

Ocean-continent comparisons

from Peter J. Smith

THE idea has spread rapidly in the Earth sciences that some segments of continent are not truly continental at all, but are of oceanic origin. One of the best known examples of supposedly 'oceanic' continent is that of ophiolite sequences which are thought to be pieces of ancient ocean floor raised to the surface and sandwiched into continental material during the collision of tectonic plates. The importance of confirming such translocations can hardly be overemphasised, not only because of the plate tectonic implications but because it would mean that some oceanic rocks could be studied without the problems of accessibility posed by a thick cover of water. So although the evidence currently available is far from negligible, it is desirable that as many more ways as possible should be found to distinguish between oceanic and continental material. It is thus unfortunate that two potential geochemical differentiators have now proved to be unsuitable.

ANOTHER recent calculation of the effect of an aerosol layer on the heat balance of the Earth has been reported by Reck (*Science*, 186, 1034, 1974). She has looked particularly at the question of whether or not a balance between heating and cooling effects can be achieved, and finds that this is only so for underlying surface albedos in the range 0.35 to 0.60. For albedos greater than 0.60, the net effect is always a warming, for surface albedos less than 0.35 the net effect is always a cooling. This effect seems to be independent of the height of the

aerosol layer above the surface, but too much should not be read into the detailed results. As Reck stresses, the calculations assume average aerosol properties, an average underlying surface albedo, and take no account of the abundances and optical properties of water clouds. Nevertheless, they bear out results of similar but more complex earlier calculations (R. A. Reck, *Atmos. Environ.*, 8, 823, 1974). Reck therefore concludes that this simpler model "is sufficient to determine both the sign and the fact of a finite limit to the heating effect"



A hundred years ago

At the last meeting of the Photographic Society a paper was read by Mr Hooper, "On the Origin, Aim, and Achievements of the Photographic Society, with suggestions as to its future development." The suggestions were, the necessity of obtaining a Royal Charter, the Society's claim upon the Government for a money grant and suitable premises, and the necessity of forming committees for scientific investigation. In the subsequent discussion, the general opinion was that there was little hope of obtaining the proposed Charter, and that it was a mistake to speak of photography as a science. "Science," one speaker said, "had done a great deal more for photography than photography had done for science."

At the meeting of Convocation of the London University on Tuesday, the motion brought forward by Mr A P Hensman "That, in the opinion of Convocation, it is desirable that women should be permitted to take degrees in Arts in this University," was, after some discussion, withdrawn from *Nature*, 11, 236, January 21, 1875

The first such 'failure' arises incidentally from a study carried out by Schwarzer and Rogers (*Earth planet Sci Lett*, 23, 286, 1974). During their investigations of the geochemistry of flow and pyroclastic rocks in Texas, Schwarzer and Rogers decided to compare the major element chemistry of all the alkali olivine basalts they could find described in the literature. The data were subdivided into continental, oceanic and island-arc sets, according to the inferred crustal environment at the time the basalts were erupted, and plotted together on $\text{Na}_2\text{O} + \text{K}_2\text{O}/\text{SiO}_2$ and AMF diagrams. Neither the major element compositions nor the differentiation trends of the alkali olivine basalts varied significantly from environment to environment.

Schwarzer and Rogers conclude first, that alkali olivine basalt is a primary magma type generated worldwide, second, that the magma is generated at sufficient depth in the upper mantle that the crustal environment above (continent, ocean or island arc) has no influence on major element chemistry, and third, that the major element chemistry of the alkali olivine basalts that reach the surface is likewise unaffected by passage through crustal material. It is thus clear that the major element chemistry of alkali olivine basalts could not be used to differentiate ocean and continent

Mitchell and Aumento (*J geophys Res*, 71, 5529, 1974), unlike Schwarzer and Rogers, were explicitly seeking an ocean-continent differentiator (for ultramafic rocks)—and with some hope of success. Previous studies had suggested that the uranium content of primary minerals in oceanic ultramafic rocks may be quite different from that of the same minerals in continental ultramafic xenoliths. Aumento and Hyndman (*Earth planet Sci Lett*, 12, 373, 1971) found that the orthopyroxene in mid-Atlantic ridge ultramafics contains 1 p.p.m. of uranium on average, whereas clinopyroxene and fresh olivine poikilitically enclosed in chrome spinels contain 0.2 and 0.03 p.p.m. uranium, respectively. Kleeman *et al.* (*Earth planet Sci Lett*, 5, 449, 1969), on the other hand, found that in the ultramafic xenoliths from the newer volcanics of western Victoria, most of the uranium is in the clinopyroxenes (0.22 p.p.m.) and the accessory mineral apatite, whereas orthopyroxene, olivine and spinels contain very little (less than 0.0049 p.p.m.).

It is now clear that these differences, though real, are misleading. Mitchell and Aumento find that average uranium concentrations in orthopyroxenes in ultramafics from ophiolite sequences range from 0.02 to 0.072 p.p.m. Not only are these values much lower than those from mid-Atlantic ridge orthopyroxenes (0.75–1.20 p.p.m.), they are significantly lower than those in orthopyroxenes from (continental) kimberlites (0.11–0.88). On the other hand, they are higher than the values for (continental) Australian lherzolite (0.002). And although the uranium content of the orthopyroxenes in the mid-Atlantic ridge rocks is high, fresh orthopyroxenes in other oceanic ultramafics have very low uranium concentrations. Similar variations occur in the clinopyroxenes. Thus, for example, the uranium content of clinopyroxenes in ophiolites are comparable to those in coexisting orthopyroxenes, but much lower than those in clinopyroxenes in ultramafic rocks from either the mid-Atlantic ridge or cratonic continental environments.

Mitchell and Aumento go on to describe even more confusing variations, but enough has been said here to show that a simple distinction between oceanic and continental rocks on the basis of uranium content is out of the question for the time being. Part of the problem seems to be that considerable uranium enrichment takes place during processes of alteration. So although orthopyroxene in oceanic ultramafic rocks is the mineral most resistant to serpentinisation, and thus whose uranium content is most likely to resemble that of original fresh rock, some alteration may nevertheless have

taken place. For example, the mid-Atlantic rocks used by Mitchell and Aumento were from 45° N to 52° N and highly altered, and it is probable that the alteration has reached the orthopyroxene on a submicroscopic scale. Other mid-Atlantic (and mid-Indian Ocean) orthopyroxenes, reported by Seitz and Hart (*Earth planet Sci Lett*, 21, 97, 1973) do have uranium concentrations which are low and comparable to the corresponding values from ophiolites. It is possible, therefore, that more use may be made of oceanic uranium concentrations when the alteration processes are more fully understood.

The uranium variations within continental ultramafics may also be due in part to differential alteration, although some may arise from different modes of formation. Nishimura (*Chem Geol*, 10, 211, 1972), for example, has suggested that uranium content may vary with depth of origin. Again, a much more thorough knowledge of the processes involved will be required before uranium content can even be considered as an ocean-continent differentiator.

Theory of the nuclear optical potential

from P. E. Hodgson

ONE of the most basic interactions in nuclear physics is that between a nucleon and a nucleus. If we know this interaction we can calculate the cross section for elastic scattering and also the distortions of the nucleon waves by the nucleus which are essential for a thorough understanding of the reactions that can take place.

This interaction is really the sum of all the individual interactions between the incident nucleon and the nucleons of the target nucleus, modified by the correlation effects due to their proximity. As it is extremely complicated, many studies have been made to see how accurate it is to replace it by a simple one-body potential that takes no explicit account of the structure of the target nucleus. This potential has a radial shape very similar to that of the nucleus itself and has a real part and an imaginary part of which the former may be thought of as refracting the incident nucleons and the latter as absorbing them. The absorption takes account of all inelastic scattering and reaction processes that remove flux from the elastic channel. The one-body potential is called the optical potential because of the similarity between the scattering and absorption of nucleons by nuclei and the scattering and absorption of light by a refracting and absorbing medium. The former can be described by a complex potential and the latter by a complex refractive index.

This simple idea has proved astonishingly successful and it is now possible to account for the elastic scattering cross sections for many nuclei at many energies by suitably choosing the parameters of the potentials. It has been developed by the addition of a pin-orbit term, which allows the polarisation of the scattered nucleons to be calculated as well. The same potentials have been extensively used to generate the distorted waves needed in nuclear reaction calculations.

One of the main difficulties with these optical potentials is that they are frequently ambiguous; that is, it is possible to find several different potentials that give equally good fits to the experimental data. These ambiguities are much reduced as the precision of the data is improved, but some of them still remain.

Thus the purely phenomenological approach to the determination of the parameters of the optical potential, in which its parameters are systematically adjusted to optimise the fit to the experimental data, is inadequate. It needs to be supplemented by a more basic calculation of the potential from the constituent nucleon-nucleon interactions. Although this is very difficult and complicated, and many approximations have to be made in order to make the computations tractable, it is nevertheless able to give valuable information on the optical potential that substantially reduces the objectionable ambiguities.

The simplest of these calculations gives the real part of the optical potential as the folding of the nucleon-nucleon interaction $v(|\mathbf{r}-\mathbf{r}'|)$ with the nuclear density $\rho(\mathbf{r})$ where \mathbf{r} is the vector to the incident nucleon and \mathbf{r}' that to a nucleon in the target:

$$U(r) = \int v(|\mathbf{r}-\mathbf{r}'|) \rho(\mathbf{r}') d\mathbf{r}'$$

Many calculations using this approach, following the work of Greenless, Pyle and Tang (*Phys. Rev.*, **171**, 1115, 1968) have proved very successful.

The imaginary part of the potential is more difficult to calculate because in principle one should take account of all possible reaction processes, but some progress has been made by considering its strength as simply given by

$$W = \frac{1}{2} \hbar \nu \rho \bar{\sigma}$$

where ν is the velocity of the nucleon, ρ the nuclear density and $\bar{\sigma}$ the mean collision cross section. This relation follows from the semi-classical theory of the optical potential.

The success of these calculations has stimulated many more detailed theories of the optical potential. Among these some very successful calculations have recently been completed by Jenkne, Lejeune and Mahaux of the University of Liège (*Physical Review*, **C10**, 1391, 1974). In this work, the calculations were first of

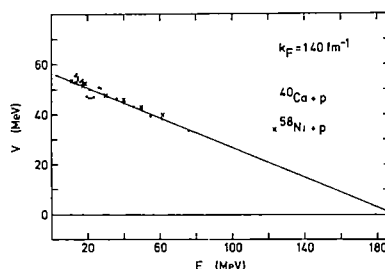


Fig 1 Calculated strength of the real part of the optical potential as a function of incident nucleon energy compared with phenomenological potentials obtained from the cross sections for the elastic scattering of protons by ^{40}Ca and ^{58}Ni

all made for infinite nuclear matter, and then the results were applied to finite nuclei using the local energy approximation. This means that we solve the problem for infinite nuclear matter as a function of its density, and then apply the results to nuclei using their known density distributions. Thus the potential at a point of density ρ is assumed to be that given by calculations of the properties of infinite nuclear matter of density ρ . This approximation is justifiable if the nuclear density changes slowly over distances of the order of the mean free path of nucleons in nuclei, which is certainly true in the nuclear interior but is more questionable in the nuclear surface. The reason for making the first calculations in infinite nuclear matter is because there the nucleon wavefunctions are plane waves, which naturally simplifies the calculations.

The nuclear matter calculations were made by solving the Bethe-Goldstone equation, which describes the interaction of two nucleons inside the nucleus. To do this calculation we need to know the interaction between two nucleons in free space, and this is known quite well from many phenomenological analyses of the scattering of protons by protons and by neutrons at many energies. Of several forms now available the Reid potential was chosen as it has already been successfully used in a wide range of nuclear matter calculations. Inside the nucleus the nucleon-nucleon scattering is modified by the presence of the other nucleons and in particular many interactions are forbidden by the Pauli exclusion principle as

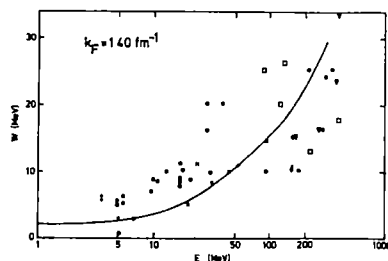


Fig 2 Calculated strength of the imaginary part of the optical potential as a function of incident nucleon energy compared with a number of phenomenological values

they proceed to states that are already occupied. This effect, incidentally, ensures that the mean free path of nucleons in nuclear matter is long enough for the shell model to be a valid description.

The solution of the Bethe-Goldstone equation and the extraction of the optical potential requires lengthy calculations, and some of the results are shown in the figures. Fig 1 shows the strength of the real part of the potential as a function of energy calculated for a Fermi momentum in nuclear matter of 1.40 fm^{-1} and taking account of the various terms in the mathematical expression for the potential. It is compared with phenomenological potentials obtained by analysing the elastic scattering of protons by ^{40}Ca and by ^{58}Ni . Up to about 100 MeV the radial dependence of the real potentials obtained in these calculations is very similar to the Saxon-Woods form:

$$f(r) = [1 + \exp\{(r-R)/a\}]^{-1}$$

used in most phenomenological analyses, but that at higher energies shows a pronounced surface peaking. Already some phenomenological indications favouring such a form have been found, and it will be important to take this into account in future analyses of the data.

The calculated energy variation of the strength of the imaginary part of the optical potential is compared with some phenomenological determinations in Fig 2. Its radial dependence changes from a predominantly surface-peaked form at the lower energies to a predominantly volume form at higher energies, and this is just the same behaviour as the phenomenological imaginary potentials. In phenomenological analyses there is much ambiguity between the amounts of volume and surface components, so that it is difficult to determine them individually. It will be a great advantage to have available some reliable theoretical guidance for the relative contributions of volume and surface absorption.

This work is in process of extension by taking into account some of the higher order terms in the solution of the Bethe-Goldstone equation, which should give improved optical potentials. Already the results obtained are most encouraging, in that they reproduce many of the features of the optical potentials already known from phenomenological analyses. As confidence in the reliability of such calculations grows, they will play an increasingly important part in guiding optical model analyses of elastic scattering cross sections. They should provide the essential behaviour of the form factors, leaving only the strength to be improved over the calculated values by optimisation of the fit to the experimental data. This should provide more reliable potentials that will improve the accuracy of the information on nuclear structure obtained from analyses of nuclear reactions.

review article

Chromosome imprinting and the mammalian X chromosome

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Chromosome imprinting is the process by which one of two genetically homologous chromosomes is predetermined to function differently from the other at a subsequent stage in development. In the coccid insects, imprinting occurs in the egg, at the time of fertilisation, it probably occurs at the same time and site in mammals, and possibly also in Sciara

DURING development, a chromosome from the father may function quite differently from its maternal homologue, a fact discovered in the 1920s by Metz¹ in *Sciara*, a dipteran insect. According to his colleagues (C. Stern, personal communication), Metz often asked, "How do the chromosomes know which parent they come from?" Almost fifty years later, this question remains unanswered. Since then, the *Sciara* system has been explored in much greater detail, and systems of differential regulation of homologous chromosomes have been discovered in two additional groups of organisms. In the 1930s, the Schraders² analysed the chromosome system of the mealybugs and other coccid insects. In 1961, Lyon³ described the system in eutherian mammals, ten years later, a group of Australian workers^{4,5} reported a somewhat different situation in marsupials. It is the purpose of this account to outline similarities among these three systems and to show that analyses of such types of chromosome behaviour provide important clues to the nature of genetic regulation during early development.

Chromosome imprinting

Crouse⁶ introduced the term imprinting to indicate the process by which differential behaviour of the members of a pair of homologous chromosomes is predetermined several to many cell generations before the stage in development at which resulting behavioural differences become obvious. She provided an operational definition of imprinting, based on her work with *Sciara*, which should suffice until the imprinting mechanisms are more clearly understood. Confusion from use of the same term in behavioural sciences can be avoided in genetics by use of the complete term, chromosome imprinting.

It is not known how, in molecular terms, the differential chromosome behaviour originates, or, having arisen, is maintained in subsequent cell lineages, but some progress is being made in showing when and where the chromosomes are subject to imprinting. In her original definition, Crouse⁶ assumed that both paternal and maternal chromosomes carry an imprint. Imprinting of only one of two homologous chromosomes is, however, sufficient to account for differential behaviour and this simpler assumption will be followed here.

In the following sections, the systems involving differential behaviour of homologues will first be reviewed as a basis for understanding the evidence available from each on the process of imprinting.

Significant features of the three imprinting systems are compared in Table 1.

The system in mealybugs

Since mealybugs and certain related coccids provide unambiguous evidence of the site and time of imprinting, this system will be reviewed first. In males, the paternal set of chromosomes becomes heterochromatic during early embryogeny and remains so in most but not all tissues during subsequent development. Meiosis is highly modified and there is no recombination. At first division, both maternal and paternal chromosomes divide equationally. At second division, the paternal, heterochromatic chromosomes are segregated from the maternal, euchromatic chromosomes. Of the four products of meiosis, only the two euchromatic derivatives form sperm, the heterochromatic derivatives degenerate *in situ*. Such heterochromatization does not occur in females^{3,7}.

The genetic inertness of the paternal set has been demonstrated by several methods, including tests with genetic markers which are expressed in and transmitted by the sons only when received from their mothers⁸. Genetic activity of the paternal set is restored, however, in those tissues in which heterochromatization is reversed during development. There is thus a strict relationship between heterochromatization and the absence of detectable gene function. Heterochromatization of the paternal set is first detectable in male embryos at about the sixth cleavage when some nuclei migrate to the periphery of the egg. This is probably the stage at which gene action begins in the mealybug embryo as the incorporation of ³H-uridine by the chromosomes cannot be demonstrated earlier⁹.

Imprinting in mealybugs

Coccid chromosomes have a diffuse kinetochore and chromosome fragments function as independent entities. After maternal irradiation, euchromatic fragments appear in male embryos, after paternal irradiation, they are heterochromatic. Thus,

imprinting itself and the process responsible for its later expression as heterochromatization are effective along the length of the chromosome¹⁰. Somewhat similar evidence has been obtained from translocated chromosomes each carrying a euchromatic and a heterochromatic segment, these chromosomes behave normally until the second meiotic division when bridging results from movement of the euchromatic and heterochromatic segments to opposite poles^{11,12}.

Evidence on the place and time in development at which imprinting occurs comes from Nur's¹³⁻¹⁵ work with parthenogenetic soft scales, a group of coccids related to the mealybug. Two distinct types of parthenogenesis were found. In one type, the second polar body substituted for the sperm to produce a diploid 'zygote'. Heterochromatization was not found in any of the four species studied. In the other type of parthenogenesis, the egg nucleus divided to produce two haploid daughter nuclei which then fused to form a diploid, homozygous 'zygote'. This type of parthenogenesis was studied in three species. In two of them, both females and males were produced parthenogenetically (type A), the males were produced toward the end of the period of egg production and apparently never functioned because there were no sperm in the numerous females examined. In the third species, functional males were produced parthenogenetically and the females arose from fusion of eggs and sperm (type B). Heterochromatization in all three species was typical. One haploid set was heterochromatized and the other remained euchromatic. In these cases, therefore, imprinting must have occurred within the egg cytoplasm and it must have affected just one of the two haploid division products of the egg nucleus, presumably during the brief period the two haploid nuclei were separated from each other. In the species in which both males and females are produced parthenogenetically, genetic factors cannot be involved because the mothers also originate by fusion of two identical haploid sister nuclei and are therefore themselves completely homozygous. Since it is very unlikely that a new mechanism would have evolved to produce the unnecessary males in the type A parthenogenetic system, the situation in the sexually reproducing species must be the same, that is, the chromosomes of the sperm must be imprinted within the egg before fusion of egg and sperm pronuclei.

All sperm of a male mealybug carry identical, maternal chromosome sets, yet both sons and daughters result. In line with the conclusions from parthenogenetic soft scales, types A and B, male embryos are produced by eggs in which paternal chromosomes are imprinted and female embryos by eggs in which they are not. Two interpretations are possible. An imprinting region could be present in some eggs, absent in others, or the region could be variable in extent, sometimes large enough to affect the nucleus in question, sometimes not. The mealybug egg is spacious enough, 0.40 mm × 0.25 mm, to permit such internal differentiation.

When present, the imprinting region within the egg seems to be restricted. It clearly does not include the egg nucleus because the maternal chromosomes are not heterochromatized in the males of sexually reproducing species. The polar bodies also apparently escape imprinting. No males, nor any heterochromatization occurred in the four examples of parthenogenesis in the soft scales in which the second polar body substituted for the sperm. In the mealybugs, the polar bodies do not degenerate but give rise to certain polyploid tissues, heterochromatization does not occur in these complex cell lineages in either males or females⁷. Further evidence that mealybug polar bodies are not imprinted comes from crosses of heavily irradiated males and virgin females, the offspring are all gynogenetic females, and originate from one or both polar bodies. Such gynogenetic females can be triploid, diploid or mosaic, but their chromosomes are always euchromatic^{16,17}.

The system in *Sciara*

There is no differential heterochromatization in *Sciara* except briefly during development of the germ line, and the paternal chromosomes are genetically active¹⁸, but differential behaviour involves entire chromosome sets in *Sciara* as it does in the mealybug.

The *Sciara* zygote contains two sets of autosomes and three X chromosomes, two of which are of paternal origin. Its constitution can be symbolised $A^m A^p X^m X^p X^p$, in which m and p indicate maternal and paternal origin, respectively, of a set of autosomes (A) or an X chromosome (X). During embryogenesis, one X^p is lost from the germ line of both sexes and from the female soma, both X^p chromosomes are lost from the male soma. The resultant constitutions are: female and male germ line, female soma, $X^m X^p$, male soma, X^m only. The somatic constitutions are thus the conventional AAXX (♀) and AAXO (♂) even though the germ lines of both sexes are AAXX. Meiosis is atypical only in the male, at the first meiotic division, the paternal chromosomes are eliminated. During the second division, the two sister chromatids of the maternal X move precociously to one pole. A sperm is formed from the single meiotic product which contains the precocious X chromatids (now chromosomes), the other products degenerate. Because all sperm contain two X chromosomes, all zygotes contain three.

Imprinting in *Sciara*

Understanding of the *Sciara* system is simplified by the presence of two types of females: AAXX' which usually produce only daughters, and AAXX, which usually produce only sons. This feature simplifies the question of the localisation of imprinting. A single male can inseminate females of the two types, in the one, the chromosomes of the sperm are altered, in the other, they are not. Yet all sperm carry the same set of maternal chromosomes. Since the behaviour of the sperm

Table 1 Cytogenetic characteristics of the three imprinting systems

	Mealybugs and related coccids	<i>Sciara</i>	Marsupials	Mammals
Type of centromere	Diffuse	Localised	Localised	Localised
Sex chromosomes	None	Soma XX(♀)-XO(♂)	XX(♀)-XY(♂)	XX(♀)-XY(♂)
Differential behaviour occurs in	Male	Male	Female	Female
Chromosome imprinted	Entire paternal set	Entire paternal set but X differentially	X chromosome	Autosome*
Parental origin of imprinted chromosomes†	Paternal	Paternal	Paternal	Paternal autosome*
Heterochromatization	Yes	No	Yes	Yes (X chromosome)*
Genetic inactivation	Yes	No	Yes	Yes (X chromosome)*
Site of heterochromatization	Germ line and soma		Soma only	Soma only
Reversal of heterochromatization may occur in	Soma		Not detected	Not detected
Meiosis	Paternal set eliminated	Paternal set eliminated	Typical	Typical

*Proposed model: an autosome is primarily affected, the X chromosome secondarily, see text.

†In typical bisexual reproduction, see text for parthenogenetic examples.

nucleus is determined by the type of egg it enters, the evidence that the fate of the paternal chromosomes is determined within the egg is incontrovertible. If imprinting does not occur within the egg but rather in the body of the father, eggs of AAXX' females must be able either to erase the imprinting or fail to facilitate its expression. The kinds of parthenogenesis seen in coccids which could provide definitive evidence that the egg is the site of imprinting have not so far been observed in *Sciara*.

By appropriately manipulating the chromosomes, *Sciara* can be induced to produce exceptional offspring, sons from AAXX' mothers, and daughters from AAXX mothers^{19, 20}. Since such exceptional offspring have the somatic chromosome complement expected of their sexes, it follows that sex is determined by the chromosomes. The genetic constitution of the mother determines only the chromosome eliminations which will occur and does not influence the sex of the offspring. The exceptional males arising from AAXX' mothers are, however, sterile, probably because their germ line constitution is AAXO rather than the normal AAXX²⁰.

The special behaviour of the *Sciara* X chromosome is determined by a controlling element located close to the centromere⁶. This controlling element is probably altered during the second meiotic division so that one of the then sister chromatids can later respond differently either to imprinting or to the later influences responsible for its expression.

In X-autosome translocations, the derivative carrying the X centromere conforms to the X-chromosome type of behaviour in its manoeuvres during development. It is reasonable to assume, therefore, that imprinting in *Sciara* need occur only at or near the centromeres.

The system in mammals

As is well known from confirmations of the hypothesis proposed by Lyon³, only one of the two X chromosomes is genetically active in somatic cells of female mammals. During early embryogeny of eutherian mammals, the active X is chosen at random and could be either maternal or paternal in each cell. In contrast, in marsupials it is the maternal X that is active and the paternal X is inactive or largely so^{4, 5, 21}. In both cases, inactivation of one X chromosome in the female serves as a dosage compensation mechanism by equating the single active X of the XX female with the single active X of the XY male. In both sexes there is the same balance between one functional X chromosome and two sets of autosomes. Selection for genes capable of functioning adequately in this situation offers a possible explanation^{22, 23} for the relative evolutionary constancy of the mammalian X in regard to both size and content²⁴. The eutherian system is selectively advantageous because random inactivation confers a 'mosaic heterozygosity' which is not possible in the marsupial system²⁵. Indeed, the apparently large effect of heterozygosity for human X-linked genes may result from mosaic heterozygosity²⁶.

The eutherian system can be derived from the marsupial system by a single mutational step²⁵. According to this scheme, the marsupial X carries a sensitive site and a receptor site adjacent to it. Imprinting of the sensitive site on the X chromosome brought in by the sperm leads to the inactivation of the site. The sensitive site on the X chromosome contributed by the egg is not so imprinted, it remains active and produces an 'informational entity' which attaches to the adjacent receptor site. At a later stage in embryogeny, the X without the informational entity (that from the sperm) becomes heterochromatic and genetically inactive, the X with the attached entity (that from the egg) remains euchromatic and active or potentially active.

To obtain the eutherian system, it is necessary to assume that during evolution a translocation moved the sensitive site from the X chromosome to an autosome and that the receptor site continued to remain on the X chromosome. Since the sensitive site is dissociated from the receptor site, the informational entity produced by the maternally-derived sensitive site would attach at random to the receptor site of either the paternal

or the maternal X chromosome. Translocation of the sensitive site to an autosome is thus the only step necessary to change the marsupial to the eutherian mode of regulation. As a consequence, in eutherians, the number of active X chromosomes would be the same as the number of maternal chromosome sets except when there is monosomy or trisomy for the autosome carrying the sensitive site²⁵.

Imprinting in mammals

What little evidence there is on site of imprinting in mammals comes from human ovarian teratomas^{27, 28} and a human diploid/triploid mosaic^{29, 30} (Fig. 1). Ovarian teratomas are usually benign tumours which resemble sexually produced embryos in that they often contain tissues such as hair and teeth. Their chromosome constitution, 46XX, is normal. No genetic markers are present in the teratomas other than those in the woman. The fact that some maternal markers are missing, however, indicates that teratomas are postmeiotic products of maternal origin. The most plausible interpretation of these data is that the abortive development culminating in a teratoma is the result of "re-entry" of the second polar body (PB2) and its subsequent fusion with the egg²⁸.

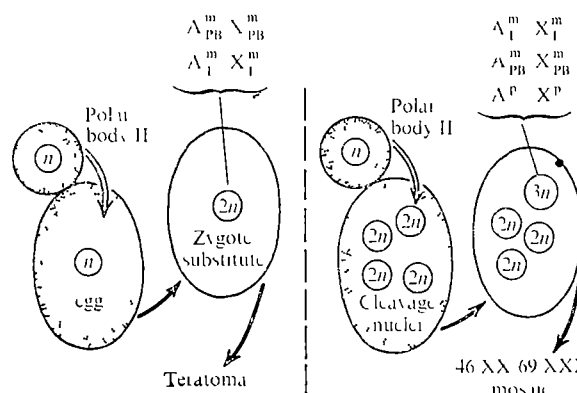


Fig. 1 Presumptive origin of human ovarian teratomas^{27, 28} (left) and a human diploid/triploid mosaic female^{29, 30} (right) and interpretation of their sex chromatin status²⁴. Stippled areas denote the regions in which imprinting would occur. A and X denote, respectively, an autosomal set and an X chromosome, superscript p indicates paternal origin and m, maternal origin, subscripts PB and E refer to polar body and egg, respectively. n, 2n, 3n denote haploid, diploid and triploid nuclei, respectively. Asterisks indicate the imprinted chromosome sets. In the teratomas, the sensitive site of one of the two haploid maternal nuclei (that from polar body II) is assumed to be imprinted, resulting in one X being active and the other inactive. Similarly, in the mosaic female, imprinting of the second maternal nucleus (again from polar body II) in the triploid cells would explain the fact that, contrary to expectation (see text), two sex chromatin bodies were found in her triploid cells.

Since the teratomas have two maternal sets of autosomes, they would, on the basis of the proposed model of regulation, be expected to have both X chromosomes active, but in fact, one X chromosome was found to be inactive (sex chromatin)^{27, 28}. The presence of an inactive X in these teratomas provides the most convincing evidence yet that the factors regulating X chromosome inactivation can be exclusively maternal in origin²³.

In the diploid/triploid (46XX/69XXX) mosaic^{29, 30}, the extra set of chromosomes was maternal in origin, but whether it was genetically identical to the maternal set in the diploid tissues is not known. The simplest explanation for the origin of the mosaicism is, again, that the haploid PB2 re-entered and fused with one of the early cleavage nuclei (diploid) to form the triploid tissues. As in normal females, a single sex chromatin body was seen in diploid cells, but two were seen in triploid cells³⁰ although two autosomal sets of maternal origin would lead to an expectation of two active and one inactive X chromosomes²⁵.

Contrary to these expectations, two maternally derived chromosome sets were associated with an inactive X in both teratomas and in the triploid tissues of the mosaic girl. In both cases, however, one of the two maternal complements was probably provided by the second polar body. It is also significant that as in the parthenogenetic soft scales (above, types A and B), the two maternal complements were presumably separated from each other before fusion. If the assumption is made that one of the two maternal complements (polar body II) was imprinted during the period of separation, then these exceptional data would no longer be inconsistent with the model²³. If, on the other hand, the maternal contribution to the individual is in the form of a single egg nucleus, then the number of active X chromosomes is the same as the number of maternal autosomal sets—whether the egg is haploid, diploid (for example because of suppression of the second meiotic division by colchicine²¹), or aneuploid for the sex chromosomes²⁵. The simplest interpretation of the mammalian data is that the imprinting zone includes all or nearly all of the periphery of the egg²³.

In three other digynic triploid/diploid mosaics (see ref. 33), both Xs of the XXY or XXYY triploid sectors were active, thus indicating that the second maternal contribution could not have been imprinted. Differences in imprinting of the maternal genome may stem from the variable behaviour of PB2, in mice PB2 may either be extruded or retained in the egg proper to form a second female pronucleus³³. In its usual place, PB2 would be subject to imprinting and, on re-entry, would provide the stimulus necessary to initiate a teratoma²⁸. If retained within the egg proper, PB2 would be imprinted only if it came in contact with the periphery of the egg before fusing with a cleavage nucleus to initiate the triploid cell lineage. PB2 has never been observed to undergo division in mammals³³, evidence for its fusion comes solely from teratomas and mosaics of the type considered here.

It therefore seems that polar bodies of mammals, but not those of coccids, may be subject to imprinting. Furthermore, determination of sex in mealybugs is by imprinting (or the two events are at least coupled) so that capacity to imprint must be variable enough to permit production of both sexes. In mammals, on the other hand, sex is determined by the chromosomes, and X chromosome behaviour proceeds according to schedule regardless of sex of the embryo, there is a single active X in both XY and XXY males²⁵.

In conclusion, (a) the egg is the site of imprinting, almost certainly in mealybugs, probably in mammals, and possibly in *Sciara*, (b) imprinting of sperm seems to be a variable process within the eggs of mealybugs and related coccids and a consistent process within mammalian eggs, (c) if two maternal chromosome sets in mammalian eggs fuse after prior separation, one may become imprinted, and (d) in both coccids and mammals there is no necessity for assuming, as may be necessary for *Sciara*, a second level of control to explain the data.

Finally, in all three genetic systems, entire chromosomes are subject to differential regulation even though only one or a few loci may be responsible for initiating differential behaviour. Immunoglobulins represent the only other case known in which homologous genetic loci function differently within a single cell³⁴. Whether this unusual behaviour—believed to be related to the function of immunoglobulins—is also based on mechanisms analogous to imprinting is not known.

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article

Ice shelves and ice flow

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New data on the Ross Ice Shelf provide an insight into the importance of the momentum with which an ice stream enters the shelf for the overall velocity field

Of the 13,800,000 km² of ice covering Antarctica, some 12,100,000 km² lie on a rock base, while about 1,500,000 km²

are in the form of floating slabs of ice round the periphery of the continent. The largest of these is the Ross Ice Shelf. In contrast to the inland ice, which built up into a vast relatively flat dome until surface slopes and ice thickness produced sufficient gravitational forces to drive ice outwards against frictional drag at the base of the ice, the floating ice shelves rest effectively on a frictionless base. The first theoretical ideas

treated ice shelves as flat plane parallel slabs of ice spreading under their own weight^{1,2}, but observations³ soon showed that ice shelves slowly increase in thickness as one moves in from the seaward boundary, known as the ice front. This thickening was satisfactorily explained in terms of drag, mainly on the sides of the ice shelves, but also by local grounding⁴⁻⁶. No theoretical studies or relevant field observations were, however, made of the corresponding problem of faster moving streams of ice within an ice shelf. This problem will now receive considerable attention as the various studies of the Ross Ice Shelf Project (RISP) develop.

The observations

To provide the first data for planning this programme, extensive airborne soundings of the ice shelf were made between

1967 and 1972. These are presented in this paper. On the basis of (a) these new measurements of ice thickness, (b) earlier measurements of velocity, and (c) the assumption of mass continuity, it is possible to estimate the direction and speed of ice flow over the entire shelf. These predictions will be tested by direct measurements of flow velocity as the Ross Ice Shelf Geophysical and Glaciological Survey operations are continued during the present Antarctic summer and the years following, as part of the RISP.

The new map of the thickness of the Ross Ice Shelf (Fig 1) is based on profiling of ice thickness by radio echo sounding from aircraft. The thin, approximately straight lines on the map show aircraft flight lines along which continuous profiles of thickness are available. These total some 35,000 km of track as compared with about 3,000 km which had been

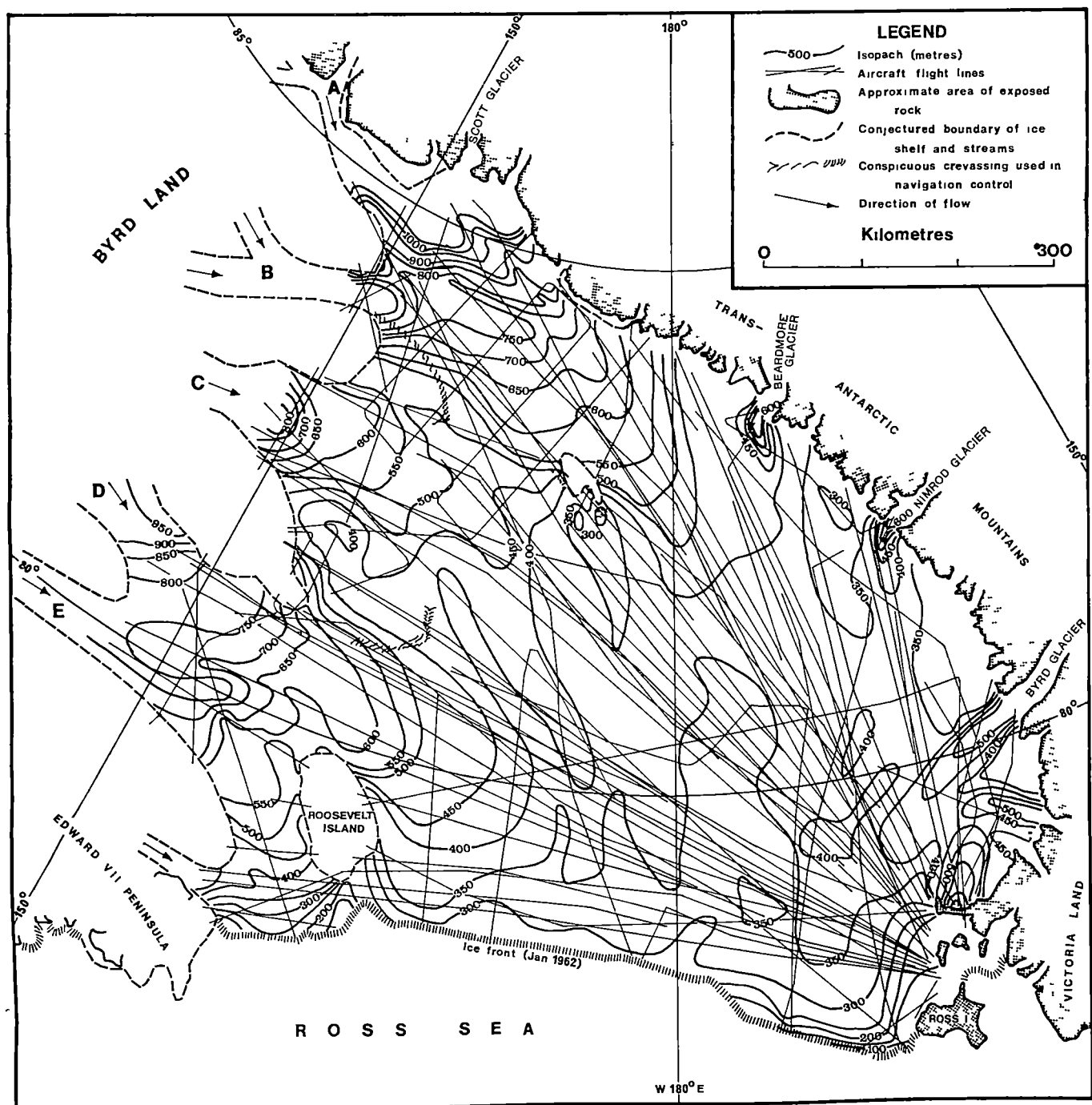


Fig. 1 Flight lines on which data were gathered

measured previously by other methods (some 50 seismic soundings plus levelling along surface traverses). The data presented in this paper were obtained as part of the joint Scott Polar Research Institute-US National Science Foundation programme of sounding the Antarctic ice sheet from long range aircraft of the US Navy experimental squadron VXE6.

Two networks of flight lines are shown on the map because some of the flights combined logistic and research tasks. Lines which are approximately radial from the southern end of Ross Island are from flights which were made to sound more distant areas or to supply fuel to Byrd station or the South Pole; lines that cut across the radial pattern are from flights flown specifically for sounding the Ross Ice Shelf.

The importance of this new information is the clearly marked distribution of ice thickness. It can be seen, for example, that the major glaciers entering the ice shelf persist for some distance as streams of thicker ice within the shelf. The possibility of deducing patterns of ice flow from distribution of thickness offers a new tool of considerable importance in the study of the flow and deformation of ice shelves.

Accuracy of the data

Before discussing the implications of the new data, we must first consider the accuracy of measurements and the related problem of aircraft navigation. The general technique of radio echo sounding has been discussed in detail elsewhere⁷⁻¹⁰. When sounding ice shelves, as distinct from inland ice, the strength of the bottom echo is generally sufficient to permit soundings from aircraft flying at their economical cruising altitude of from 7,000 to 10,000 m, as opposed to the lower altitudes necessary for sounding of inland ice. A time delay on the oscilloscope trigger, plus an appropriate sweep speed, was used to spread the echoes from top to bottom of the ice shelf over a substantial portion of the oscilloscope screen, and hence of the recording film. Under these circumstances, errors of measurement of ice thickness are of the order of 10 m, of which 4 m are due to scaling off the film record and about 1.5% due to uncertainty over the velocity of radio waves in ice and firm.

An overall test of accuracy comes from comparison of thicknesses interpolated from Fig. 1 with those at 44 locations measured by seismic sounding¹¹. The arithmetical mean difference with regard to sign was only 4 m, the radio echo depths being the greater. The root mean square deviation between the two sets of measurements was 25 m and the maximum difference 67 m. These results indicate that the main sources of error probably come from errors of navigation and interpolation, since both are likely to produce random errors which will cancel out when averaged over many observations. The remaining difference of 4 m between the two systems is not significant.

Navigation was the chief difficulty. Most of the flight lines in Fig. 1 were flown during the 1969-70 season in a C130 (Hercules) aircraft at 7,000 to 10,000 m. Conventional navigation methods (TACAN, Sun shots, radar fixes, air photographs when within 150 m of mountains) were used. Although errors of up to 50 km may be expected, careful navigation by the aircrew, subsequent checking of interpolated (dead reckoning) positions and the relatively slow variation of wind speed and direction in the upper troposphere in summer, all served to limit errors. After some flight lines were rejected because of inconsistencies of navigation, the selected lines were plotted in a series of colours along each line, each segment of colour corresponding to a given 50 m range of depths. Then, since the remaining navigational errors were not large, a clear pattern of colour emerged on which isopachs (lines of equal thickness) could be drawn with confidence. Variations of thickness between different flight lines indicate that naviga-

tional errors were generally less than 16 km. Where there was a high density of flight lines, averaging of navigational errors should reduce errors in the position of isopachs to 10 km or less.

In 1971-72, inertial navigation (Litton 51 C) fitted to the aircraft gave a continuous record of position to an accuracy of better than 5 km for two flights that were made partly over the ice shelf. On one of these flights, four parallel lines that were flown over the south-eastern corner of the ice shelf did much to resolve the detailed pattern of thickness, and subsequently of ice flow, in this key area.

Results of four flights in 1967 by C131 J (Super Constellation) aircraft are also included. These were made at lower levels (1,000 to 2,000 m) where winds are more variable and navigational errors greater. The flight paths have been adjusted to match the thickness data from these flights to those obtained during the 1969-70 season.

Thickness pattern and streamlines of flow

Figure 1 shows the extent to which glaciers persist as streams of thicker ice within the shelf. The same effect is seen in relation to the streaming of thinner ice, especially downstream of the small ice rise around 83°S, 172°W. It is possible that the thinner stream of ice starting around 82°S, 164°W may also be caused by local grounding in this area, even though no ice rise is present. Alternatively this thinner stream may be the result of shear between ice streams moving at different speeds. Similar thinning is seen around 84.5°S, 155°W between ice streams A and B and between ice streams D and E around 80.2°S, 155°W.

In the central part of the ice shelf, where the ice shelf varies slowly between 450 and 300 m in thickness, the isopachs are less accurate, and it is more difficult to deduce streamlines. The isopachs in this area represent mean values from a number of flight lines, especially for the closed isopachs for 350 and 400 m between 170°E and 180° in which most but not all ice thicknesses exceed the indicated values. Near the ice front the effects of bottom melting appear to dominate the thickness pattern, especially in the vicinity of Ross Island, where rapid melting has been shown to take place^{11,12}.

It is clear that in order to deduce directions of ice flow, we must use these indications of streaming, rather than attempt to use mean surface slopes as is done in the study of inland ice. (In the case of floating ice shelves, surface slopes are proportional to thickness gradients.) Nevertheless, there is a general decrease of ice thickness as one moves along individual flowlines towards the ice front, and even in the few areas in which the ice thickness does increase towards the ice front, the rate of increase is slow and is probably due to new accumulation along the line of flow rather than compression of ice in this direction. Indeed there is little evidence from the map that compression along a flowline can take place, although compression occurs normal to flowlines over much of the ice shelf. This agrees with the concept that ice shelves spread out under their own weight while floating on a frictionless base^{1,2}.

The increasing thickness of ice shelves with distance from the ice front, which is explained by drag at the lateral boundaries of the shelves, has a counterpart in individual ice streams within an ice shelf. When a rapidly moving stream enters the shelf, it will be retarded by the slowly moving ice on either side, with the result that its thickness increases in the upstream direction. But in the ice to either side the opposite effect takes place, the rapidly moving ice stream drags the surrounding ice forward, causing a thinning as one moves upstream. This is particularly noticeable between Beardmore, Nimrod and Byrd Glaciers. We will consider this point later in more detail.

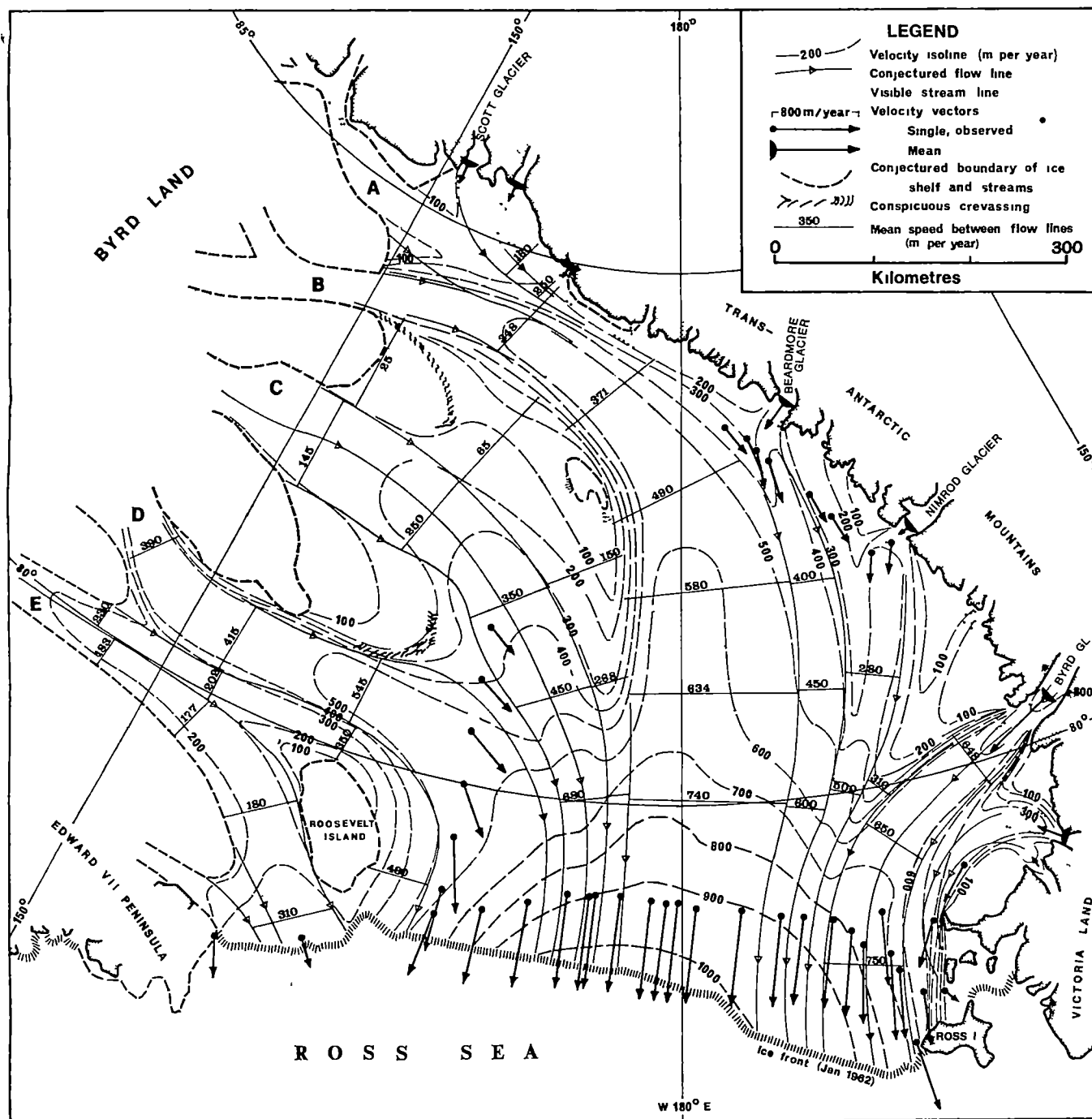


Fig. 2 Velocity field of ice flow

Velocity distribution within the Ross Ice Shelf

Figure 2 shows the speed and direction of movement over the Ross Ice Shelf. Vector arrows show direct measurements of movement made by earlier investigators¹⁴⁻¹⁶. Streamlines of ice flow deduced from the thickness pattern and from calculations are shown by continuous lines, while dashed lines indicate lines of equal speed. Between 180° and 150°W streamlines were deduced entirely from the thickness pattern, and their direction agrees well with the direct observations of movement along 164°W.

We have assumed mass continuity of flow to extrapolate velocities upstream from Dorrer's velocities along 79°S (ref. 16). If \bar{V}_1 is the known mean velocity along a line 1 be-

tween two flowlines, the mean velocity \bar{V}_2 along line 2 between the same flowlines at a distance δx upstream is given by

$$\bar{V}_2 = \bar{V}_1 \frac{\bar{H}_1 W_1}{\bar{H}_2 W_2} - \bar{B} \frac{\delta x}{\bar{H}_2} \left(\frac{W_2 + W_1}{2 W_2} \right) \quad (1)$$

where \bar{H}_1 and \bar{H}_2 are the mean ice thicknesses and W_1 and W_2 the widths between the flowlines along the lines 1 and 2, and \bar{B} is the mean mass balance per unit area over the area bounded by lines 1 and 2 and the flowlines. This mass balance must take account of accumulation and ablation on both upper and lower surfaces of the ice shelf. Observations by Cray and coworkers¹¹ show the distribution of net annual accumulation on the upper surface of the ice shelf, which

varies from 0.16 to 0.30 m yr⁻¹ of ice. We know that bottom melting up to at least 0.50 m yr⁻¹ occurs near the ice front while freezing may take place under the southern part. We assumed bottom melting of 0.12 m yr⁻¹ between 79°S and 80°S, falling off to zero between 83°S and 84°S, and we have made no allowance for any bottom freezing. The resultant mean velocities (m yr⁻¹) are shown in Fig. 2 above the lines to which they apply.

Although streamlines could readily be deduced from the thickness of the ice on the shelf between 150°W and 180°, between 180° and 160°E their location was less clear. In this section, flowline positions were adjusted through a process of iteration until calculated velocities near the Beardmore, Nimrod and Byrd Glaciers matched the observations.

The velocities between the pair of flowlines finishing either side of the 180° meridian are worthy of particular attention. Once the streamline flow in the south-eastern corner of the ice shelf had been determined by the 1971–72 flights with inertial navigation, it was clear that the major discharge of ice stream B passed to the south of the ice rise at 83°S, 172°W. As we knew the mass discharge of the Robert Scott Glacier and the Amundsen Glacier to the west, and could identify their flowlines to 165°W, we estimated the mean velocity of their stream at this point as 250 m yr⁻¹ compared with a mean value over the line also including the discharge of stream B of 348 m yr⁻¹. The velocity of the latter must therefore be around 500 m yr⁻¹. This result is particularly interesting for it explains why the rapid discharge of the stream B, which is only 800 m thick where it enters the ice shelf, dominates and rapidly bends the flow from the Robert Scott and neighbouring glaciers, although they are several hundred metres thicker on entering the ice shelf. In this area where there are strong thickness gradients, and hence greater surface slopes than elsewhere, we see that the slopes do not govern the movement. This is controlled mainly by the momentum of the stream with the largest mass discharge into the ice shelf.

Although a fully satisfactory three-dimensional solution to the problem of deformation within ice shelves has not yet been given, some relevant factors can be appreciated by considering the equations for quasi-static creep of an ice shelf. We take the x axis to be oriented in the direction of flow and the z axis vertical. Then provided we are clear of local boundary effects and have no friction on upper or lower surfaces, we assume the shear stresses in the xz and yz planes to be zero, so the basic equilibrium equations⁶ reduce to

$$\frac{\partial \sigma_x}{\partial x} + \frac{\partial \tau_{xy}}{\partial y} = 0 \quad (2)$$

$$\frac{\partial \sigma_y}{\partial y} + \frac{\partial \tau_{xy}}{\partial x} = 0 \quad (3)$$

$$\frac{\partial \sigma_z}{\partial z} = \rho_i g \quad (4)$$

where ρ_i is the density of ice and g the gravitational force. The earliest theories of the flow of ice shelves^{1,2} were based on (4), while solutions including the effect of lateral drag either used the simplifying assumption⁵ that $\partial \tau_{xy}/\partial x = 0$, or⁶ that $\partial \tau_{xy}/\partial x$ was constant. We can see from equation (2) that as an ice stream is slowed down as it moves out into a uniform ice shelf, the change of stress in the direction of flow, and hence by simple theory of the ice thickness, must be balanced by a gradient of shear stress in the normal direction.

Similarly, any horizontal stress gradient normal to the flowline, which we expect to be present if the thickness varies across an ice stream, must be balanced by a gradient of shear stress in the flow direction.

We can summarise the position by saying that if an ice shelf is of uniform thickness, no differential stresses $\partial \sigma_x/\partial x$, $\partial \sigma_y/\partial y$, $\partial \tau_{xy}/\partial x$, $\partial \tau_{xy}/\partial y$ will be present. If the thickness varies in the flow direction, but is constant in a direction normal to flow, then $\partial \sigma_x/\partial x$ and $\partial \tau_{xy}/\partial x$ are not zero, and approximate solutions^{5,6} apply. But we must take account of all the four stress gradients if we are to obtain a solution to the problem of differential flow within an ice shelf.

Our results show that the largest thickness gradients occur normally to the flow direction on the sides of the major ice streams, and hence it seems that our maximum horizontal stress gradients $\partial \sigma_y/\partial y$ also occur here. From equation (3) we see also that the expected maximum values of $\partial \tau_{xy}/\partial x$ are in these regions. This fits the physical picture that the drag on the sides of an ice stream should vary most rapidly near its entry to the ice shelf. Although we cannot give a full solution, we see that as the shear gradually disappears as one moves further into the ice shelf, the thickness of the shelf becomes more uniform. Then, if we neglect other factors such as bottom melting and drag at the boundary of the ice shelf, we expect that as the ice stream approaches the same thickness as the ice shelf on either side, differential motion within the shelf will become small.

Our results indicate that the momentum with which an ice stream enters the ice shelf is perhaps more important than expected. The same process, but in an inverse sense, appears to explain the thicker ice to the south of Minna Bluff, the promontory that juts out into the ice shelf around 79°S to the south of Ross Island. But in this case it is the ice shelf streaming past the promontory that seems to provide the force that, rather than lateral drag, causes thickening in this area. The most complex situation is that caused by different ice streams entering the south-eastern corner of the ice shelf from different directions and at different speeds. Clearly a full solution for this case, both analytically and in terms of gathering adequate field data on all boundary conditions will be difficult. Similar considerations also apply near the ice front where the mechanics of calving of large tabular icebergs must depend on the same basic equations.

The value of the present work lies in the way in which the new data draw attention to these problems. Although predictions of the velocity of movement over the whole area of the ice shelf help our understanding of the region, the direct measurements of velocity that will be made in the near future by use of satellite position fixing methods will provide an interesting test of our main conclusions.

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letters to nature

Outburst in U Cephei

A SYSTEMATIC survey of the profiles of the H α line in binary stars, shell stars, Be stars and related peculiar objects is being conducted by us (and G J Peters) with the Varo image intensifier attached to the coude spectrograph of the 120-inch telescope and the 24-inch coude auxiliary telescope of the Lick Observatory. Emission at H α is found to be a more common phenomenon among the Algol-type eclipsing binaries than was thought before. Usually, but not always, the emission is best observed during the primary minimum when the light of the hotter and brighter component is either greatly reduced or completely occulted. This is in agreement with the models representing the Algol systems as evolved binaries in which mass is still being transferred from the late-type secondary component to the hotter primary star^{1,2}. Although the general picture is fairly clear, the problem remains how the on-flowing material and its angular momentum are distributed among the primary star, the disk revolving around it and a possible envelope enclosing the whole system. The purpose of our survey is to furnish quantitative data on this process.

Observations of U Cephei show that the mass transfer and disk formation may be a very variable process. Batten³ and coworkers have tried to detect emission in this system, but found either no emission or at best a marginal phenomenon. On August 8, 1974, however, we observed very strong H α emission during a primary eclipse. Photometric observations by L. McDonald accurately determined the time of mid-eclipse (1974 August 8 4345 = JD 2442267 4345 heliocentric) and the phases.

Our equipment gives a very good dispersion (17 Å mm⁻¹) and good time resolution (exposure times were 5 to 20 min), so that the behaviour of the emission line can be studied in detail. Figure 1 shows the density tracings of nine consecutive spectrograms and Table 1 gives the peak intensities of the violet and

red emission lobes as functions of phase. Our timing of the exposures was very fortunate: the first plate coincided with the beginning of the total eclipse, the third plate with mid-eclipse, and the fifth plate with the end of the total phase. Five more spectrograms were obtained in the ensuing partial eclipse. All the plates show a very strong, deep and relatively narrow central absorption core at H α . During and near the total phase, this core, and several weaker absorption lines, were the result of the secondary (G8 III–IV) component. Emission lobes appear on both sides of the H α absorption core. Contrary to the Victoria observations made in September, however, no other emission lines were observed, although some suitable lines of Fe I, Fe II, and He I do lie in the observed spectral region around H α .

Table 1 Peak intensities of emission lobes

Plate	Phase d	Fraction of period (P)	Peak intensity (continuum = 1.00)	
			Violet lobe	Red lobe
EC 12579	−0.0302	0.9879	1.07	1.49
12580	−0.0135	0.9946	1.13	1.33
12581	0.0012	0.0005	1.20	1.22
12582	0.0200	0.0080	1.33	1.15
12583	0.0337	0.0135	1.35	1.09
12584	0.0464	0.0186	1.30	1.07
12585	0.0573	0.0230	1.28	1.06
12586	0.0686	0.0275	1.20	1.05
12587	0.0803	0.0322	1.10	1.01
12588	0.0873	0.0350	1.02	1.00

At the beginning of the total phase, the red emission lobe is very strong, while the violet lobe is marginal but real. As the eclipse advances, the red lobe weakens, while the violet lobe grows in strength. Both are equally strong at mid-eclipse, and the H α profile is symmetrical. The violet lobe reached its greatest intensity near third contact, but never quite matched the peak intensity of the red lobe on the first plate. This suggests greater accumulation of material on the impact side of the primary star. As the primary component gradually emerges from the total eclipse after phase 0.02P, both emission lobes decline in intensity, but the violet lobe remains stronger.

Qualitatively, this sequence of events agrees with the model of a rotating ring around the primary component, originally proposed by Joy⁴ for RW Tauri. For the first time, however, the dispersion and time resolution have been so good as to permit a quantitative analysis of the profiles, which is under way.

This complete ring—or better disk—must have been formed very recently. Previous observations³ indicate only a 'bridge' of material between the two components⁵, while Huang and Struve⁶ predicted that emissions in U Cephei and a few other similar systems should occur intermittently. It is probable that the primary cause was an outburst of the secondary component, with the ensuing increase in the rate of mass transfer. If so, photometric timings of future minima should show a jump in the period.

We suspect a real change in the structure of the disk within the month that elapsed between our observations and those by Batten *et al.*⁷ The seven-colour intermediate-band photometric observations made by L. McDonald simultaneously with our spectrographic observations show distortions in the light curve near the onset and the end of the total eclipse. The duration of

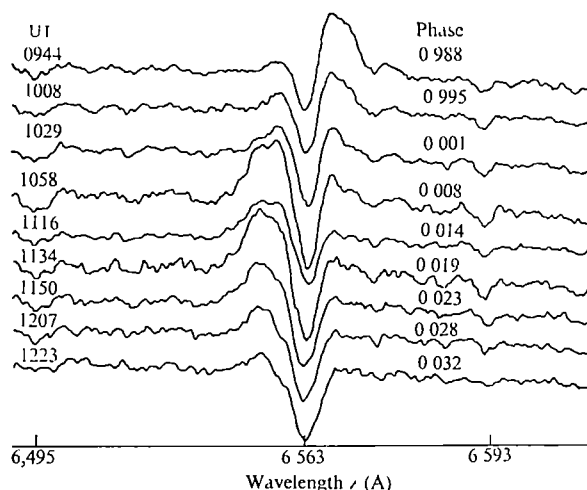


Fig. 1 Density tracings of the H α region on nine spectrograms taken on August 8, 1974. Tracings are superimposed so that the absorption cores nearly coincide. Laboratory wavelengths in Angstroms are indicated on the λ axis.

the total phase seemed to be much shorter than usual, but in most colours this cannot be simply interpreted as the consequence of an increased photospheric radius of the primary star. Rather, one must invoke a 'third light' in the system. Somewhat similar but weaker bumps in the light curve of RW Tauri observed by Grant⁸ were interpreted by him as the light of the emission lines. Line emission cannot be responsible for the entire effect in U Cephei.

Outbursts of this type may be fairly frequent in the Algol-type binaries, and their observations should contribute greatly to our understanding of the mass transfer processes in close binary systems.

We thank L. McDonald for preliminary information on his photometric observations, H. L. Burger for assistance, and R. C. Crawford for the Figure.

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Outburst of U Cephei

We believe that our recent observations of the eclipsing binary U Cephei have implications for the study of the evolution of binary systems and the understanding of novae.

The system U Cephei contains a B7 V star of mass $4.2 M_{\odot}$ and radius $2.9 R_{\odot}$ and a G8 III-IV star of mass $2.8 M_{\odot}$ and radius $4.7 R_{\odot}$. The radius of the circular orbit is $14.7 R_{\odot}$, and the orbital inclination is close to 90° (ref. 1). The orbital period of approximately 2.5 has been increasing throughout the nearly 100 yr that the star has been recognised as an eclipsing binary. The average rate of increase is about 5 parts per 10^6 per cycle, but there are irregular abrupt period changes superimposed on the average increase, and the present rate of increase is faster. The average rate can be explained as a result of transfer of matter from the G8 star to the B7 star, and is one of many pieces of evidence for the existence of gas streams in this system.

During the total eclipse of the primary star, in systems of this sort, one can often observe emission lines superimposed on the spectrum of the secondary star. These lines originate in the gas streams, but in spite of the strong evidence for such streams in U Cephei, emission of this kind has been reported in the spectrum only once², and that was very weak. During the eclipse of September 7, 1974, however, very strong emission was observed by one of us (B. W. B.) in the course of a routine programme of observation of this and similar systems. The emission was clearly present in all Balmer lines from H β to H18 (Figs 1 and 2b), and also in the H and K lines of Ca II, $\lambda 4481$ of Mg II, and possibly in some lines of Fe II and He I. The changing intensities of the red and violet components during the course of the eclipse suggest that the emission arises in a disk that rotates around the primary star with an average velocity close to 250 km s^{-1} (Fig. 1). This emission persisted and was seen during all

the observable eclipses in September and early October. By October 17, however, it had appreciably weakened. During the same time, emission was visible at H α even in full light. For about a week in the middle of September this H α emission was accompanied by a sharp absorption component, which could also be seen at H β , H γ , and H δ , with a displacement to the violet corresponding to a velocity of several hundred km s^{-1} (Fig. 2a). This component shows large variations, on September 15, about 0.25 P after primary minimum, its displacement corresponded to -620 km s^{-1} , on September 16 at 0.67 P to -350 km s^{-1} , on September 21 (at almost the same orbital phase) to -250 km s^{-1} , and on September 22 (at the end of primary eclipse) to approximately -700 km s^{-1} . The disk around the primary star seems to have been expanding, and some matter probably left the system. During September the spectrum of the primary star changed, lines of He I almost disappeared, while those of Ca II, Mg II, and Si II became stronger. Lines of Fe II previously

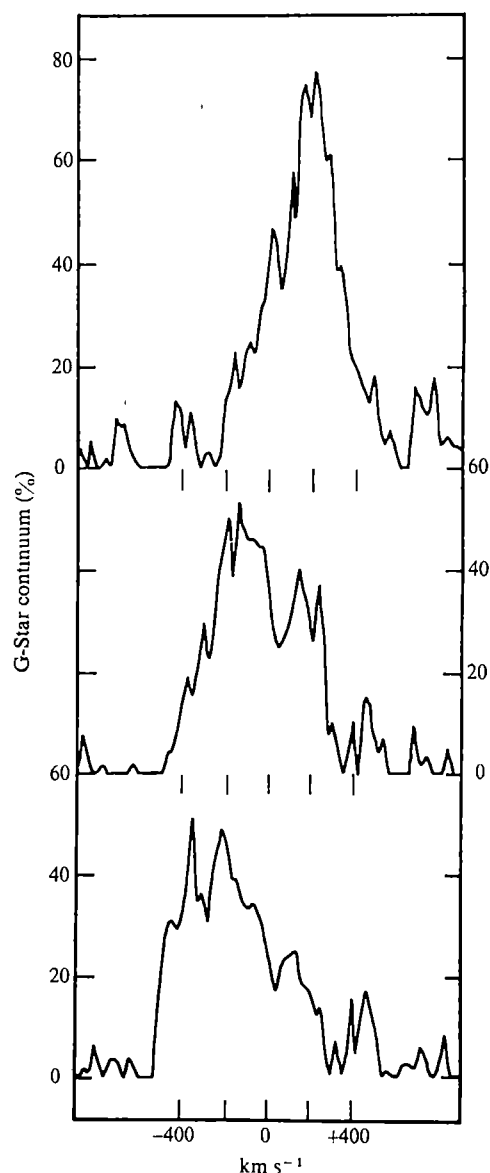


Fig. 1 The change of emission intensity at H β with phase during the eclipse of September 7, 1974. The normal emission-free G-type spectrum has been subtracted from the observed spectra. Exposure times for each plate were about 45 min. The upper (first trace) was centred about 1 h before, the second (middle) about 10 min before, and the third about 40 min after the estimated time of mid-eclipse. The horizontal scale, referred to the rest position of H β is approximate.

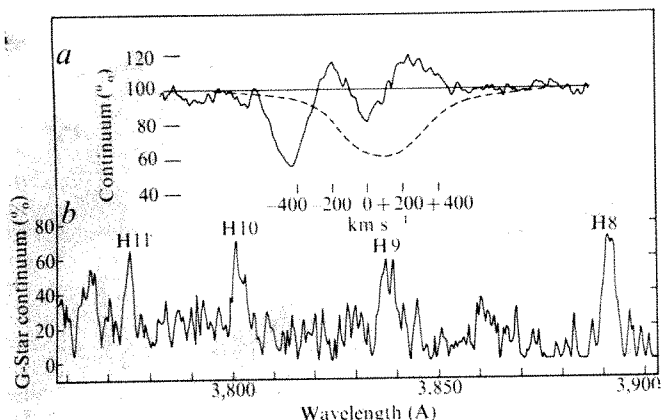


Fig. 2 *a*, Rectified intensity tracing of the line profile of H α on September 16, 1974, referred to the continuous spectrum in full light. The zero of the velocity scale is the approximate rest position of H α . The dashed symmetrical profile is the normal absorption profile of H α displayed by an amount appropriate to the orbital phase. The displacement of the violet absorption, over 400 km s⁻¹, is greater than the amount quoted in the text which is the mean for the lines H α , H β , H γ , and H δ . *b*, Rectified intensity tracing of a region of the spectrum of U Cephei made from a plate obtained about one hour before mid-eclipse on September 7, 1974. The normal emission-free G-type spectrum has been subtracted from the observed one, but there is still some contribution from the B-type spectrum. The wavelength scale is approximate.

invisible appeared in the spectrum; especially prominent amongst them is the triplet $\lambda\lambda 4924, 5018, 5169$ which is normally barely visible but which was also seen in October 1969 when emission was last observed in the system. The spectral type is more like B9 or A0 (the original Henry Draper type) than the normal B7. Photometric measurements out of eclipse in late September showed that the $(B-V)$ colour of the primary star had changed to 0.0 mag from its customary value of nearly -0.1 mag. The $(U-B)$ colour, however, had not greatly changed.

Photometric observations during eclipse show a changed form of light curve that has also been observed by Plavec and Polidan³ and by Olson⁴ and which is illustrated in Fig. 3. We believe that the abrupt change of slope during ingress is real, but we cannot yet explain it. Note that totality is shorter than normal, but the duration of the whole eclipse seems longer than usual. During totality, the V magnitude and $(B-V)$ colour of the secondary star are normal (as is also the secondary spectrum). The $(U-B)$ colour is different; the secondary component has always shown an ultraviolet excess (compared with other G8 III-IV stars) of about 0.1 mag. During this outburst the excess has been up to 0.2 mag greater, that is $(U-B) = 0.25$ mag. We believe that this is a decisive argument against ascribing the excess to metal deficiency of the secondary star. There is some indication that the system as a whole is slightly fainter than usual out of eclipse, but the difference does not exceed 0.05 mag, and because our photometric system is not precisely the U, B, V system we are not sure that the difference is real.

The key to our interpretation is that although totality is shorter than usual, during the total phase nothing except the U magnitude is different. The secondary star, therefore, is in its normal state. If the primary star has formed a new effective photosphere, appreciably larger than the normal one, then both the decrease in the duration of totality and an increase in that of the whole eclipse would be inevitable. The new photosphere would have to have a lower temperature than the old one did, because the out-of-eclipse brightness of the system has not greatly changed. The duration of totality differs from eclipse to eclipse, and even from colour to colour at the same eclipse. On September 12, totality lasted about 50 min (0.014 P) instead of just over 2 h (0.037 P)—the normal value. Since the sine of the

phase angle of second contact is given by the difference in the radii of the two stars divided by their separation, and the radius of the secondary star is apparently unchanged, these figures imply a radius for the effective photosphere of 4.1 R_{\odot} —an increase of 42%. If the radiating surface is to increase by this amount without changing the total luminosity of the system, its temperature must be decreased by a factor 0.84. According to Morton and Adams⁵, a B7 V star has an effective temperature of 13,600 K, and this must therefore be reduced to 11,400 K—appropriate to a spectral type between B8 and B9 and not inconsistent with the changes observed in the spectrum and the $(B-V)$ colour. The proposed effective photosphere could have been created either by an exceptional ejection of matter from the G8 star, or by an expansion of the outer layers of the B7 star. We incline to the latter possibility because of the evidence for the expanding shell, and the apparent normality of the G8 star. The instability of the B7 star may, however, have been triggered by an earlier unobserved eruption of the G8 star.

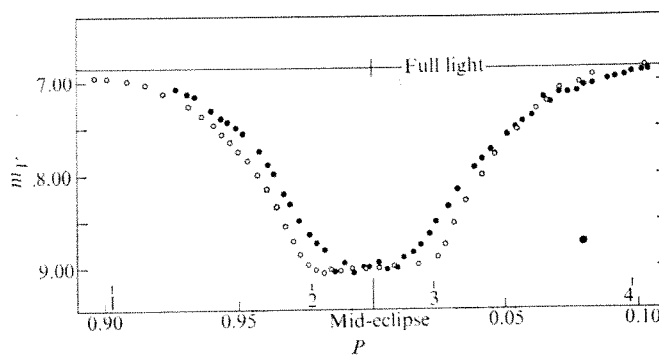


Fig. 3 Photometric observations of U Cephei with a V filter on September 27, 1974 (filled circles) compared with normal points derived from similar unpublished observations by Broglia in 1957-8 (open circles). Observations from Victoria in 1971 and 1973 fit Broglia's light curve perfectly, but do not cover it completely. Times of the four contacts for Broglia's curve are indicated on the time axis. The times of mid-eclipse, determined separately for each light curve, have been made to coincide. The line indicating full light at 6.85 mag refers to Broglia's curve. Note that even the undisturbed light curve is asymmetric.

Our observations and those of ref. 3 show that U Cephei has been active throughout August, September, and part of October 1974. Spectral scans made in early July by Rhombs (private communication) suggest that some changes were taking place even then. Photometric and spectroscopic observations by us in September, 1973, showed the system to be normal and without emission then. The photometry was confirmed at the end of September, 1973, by Coyne⁶. So the outburst could not have begun earlier than October, 1973, nor later than July, 1974. Our most recent observations suggest that it is ending in October, 1974: the emission is weaker, the lines of ionised metals are disappearing from the primary spectrum, and the light curve has more nearly its usual shape. We believe we may have observed the mechanism of an abrupt period change in progress. Bakos and Tremko⁷ have reported a much smaller distortion of the light curve that they observed in August, 1969, a few weeks before emission was first detected in the spectrum of U Cephei. Hall⁸ has also reported large changes in the light curve of RW Persei. The detailed spectroscopic and photometric coverage of this incident by several independent groups is so far, however, unique in the study of close binary systems.

Further study of our observations will undoubtedly improve our understanding of the process of mass transfer in systems of this kind. We also believe it will have implications for our understanding of novae. The expanding shell and the larger and cooler

temporary photosphere are found in novae. The binary nature of many ex-novae has been demonstrated, and it is generally accepted that nova eruptions are in some way linked to the presence of a close companion. Most theories are based on the assumption that the seat of the eruption is a degenerate star (see ref. 9). The present observations suggest that something similar can take place in a system containing a main-sequence star.

We thank the observers who helped to obtain spectrograms.

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High-frequency radio observations of the Stephan's Quintet region

RADIO observations have been reported for several apparently interacting astronomical systems¹⁻⁴. These observations do not provide conclusive data as to the frequency of occurrence of radio emission from multiple systems compared with that for normal galaxies. Although the detected radio fluxes do not generally fall in the 'radio galaxy' range, there is evidence that for some interacting systems at least, there is a statistical excess of the number of radio sources in their vicinity^{5,6}. The aperture synthesis observations mostly show sources coincident with member galaxies; a few show emission from regions between the galaxies. In the case of Stephan's Quintet, observations⁷ with the Westerbork Synthesis Radio Telescope (WSRT) at 1.415 GHz suggest an arc-shaped source partially encircling a point source centred on NGC7319.

The National Radio Astronomy Observatory three-element interferometer was used at frequencies of 2.695 and 8.085 GHz, between January 1 and July 3, 1973, to synthesise areas of 18' × 18' and 6' × 6' respectively, centred at $\alpha = 22^{\text{h}} 33^{\text{m}} 48.0^{\text{s}}$, $\delta = 33^{\circ} 41' 00''$ (1950) near Stephan's Quintet. Observations were obtained at 16 telescope spacings from 100 to 2,700 m. The resulting synthesised beamwidths at the two frequencies are 9" by 6" and 3" by 2" respectively, (both at position angle 324°), with the close-in side lobe levels less than 30%.

In the present observations, the point source of ref. 7 is detected at both frequencies but appears somewhat extended. This source was successfully subtracted from the synthesised map by a model at 2.695 GHz composed of a 10.4 mJy point source, with a 3.1 mJy slightly extended component 1" east

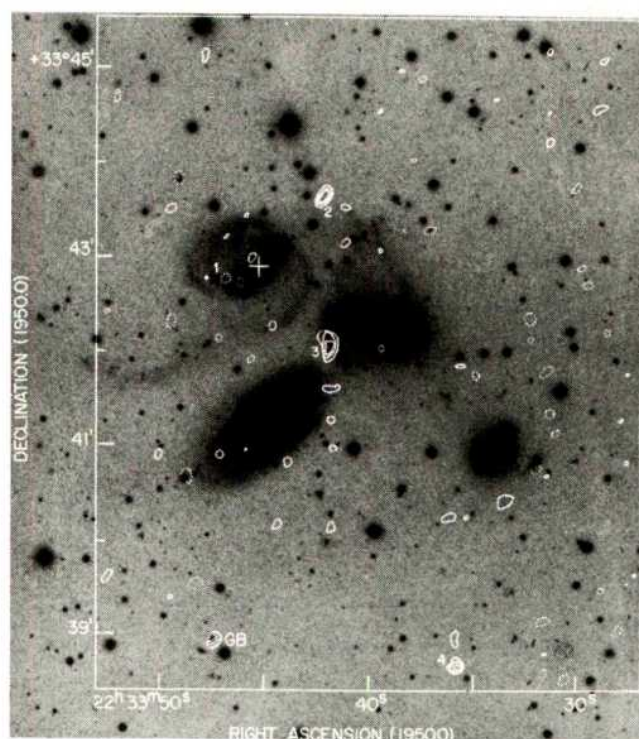


Fig. 1 The 2.695 GHz observations are superimposed on a 200-inch print of Stephan's Quintet. The cross represents source No. 1 which has been subtracted from the map. The synthesised beam is given in the lower left corner. The outer 2.695 GHz contour level is at 1.2 mJy (1 r.m.s. = 0.5 mJy) increasing thereafter in intervals of 0.8 mJy.

and 3" south of it. The same model with fluxes of 2.5 and 1.3 mJy respectively, fits the 8.085 GHz observations; the latter flux is more than four times the root mean square flux at that frequency. In the subtraction procedure used, no single point source in the vicinity sufficed to remove the observed source; the two component model was the best at minimising the residuals. This indicates that the source is definitely extended. Furthermore, the crescent-shaped source is clearly resolved at the lower frequency into two extended sources, roughly coincident with the peaks seen in the map of ref. 7. Both sources are below the detection limit at the higher frequency.

In Fig. 1, we present the results of our 2.695 GHz observations, superposed on a 200-inch photograph provided by H. C. Arp. This superposition is based on an overlay program for the Palomar Sky Survey, which, because of the small scale of the latter, can result in a displacement error as large as 15" in spite of the much higher positional accuracy of the interferometric observations. As in the WSRT map, our source No. 1 is centred on NGC7319. Source No. 2, corresponding to the northern source of the crescent of ref. 7, seems to lie on a blank field. But source No. 3, the southern source, falls quite near the HII emission features seen by Arp⁸ in the spiral arm of NGC7318b. Source No. 4 seems to be quite near a small faint compact galaxy. The slight discrepancy between our positions for sources 2 and 3 and those shown on the overlay of ref. 7, can be explained by the displacement error.

Table 1 Source data

Source	RA (1950.0) (±0.19 s)	Dec. (1950.0) (±2")	$S_{2.695}$ (mJy) (±0.5)	$S_{8.085}$ (mJy) (±0.3)	$\alpha_{1.400}^{2.695}$ (±0.3)	Remarks
1	22 h 33 min 45.9 s	+33° 42' 57.8"	13.5	3.8	1.1	Source on nucleus of NGC7319
2	22 h 33 min 42.2 s	+33° 43' 39.9"	3.2	<1.5	<1.7	Source within the crescent
3	22 h 33 min 42.2 s	+33° 42' 02.9"	5.1	<1.5	<1.7	Source within the crescent
4	22 h 33 min 35.4 s	+33° 38' 54.0"	5.5	—	1.5	Out of 8.085 GHz field of view

Our observations do not reveal the arc structure seen in the WSRT map. In order to examine the possibility that the arc may have resulted from a blending of our two sources in the lower resolution map, we have convolved our observed map with a larger beam approximating the WSRT resolution. The resulting map still showed the two sources well resolved. We conclude that the apparent discrepancy results from either of two possibilities. Firstly, that such an arc does indeed have a continuous distribution as seen in the WSRT map, but that the continuity is broken at our higher frequency by the weakness of the emission at all areas other than the peaks. If the whole arc structure has as steep a spectrum as the two sources, (as seen in Fig. 2), then the flux density level received from the rest of the arc would never exceed three times our r.m.s. level. Secondly, the structure may be a blend of four or more sources, of which only two are within our detection limit at 2.695 GHz.

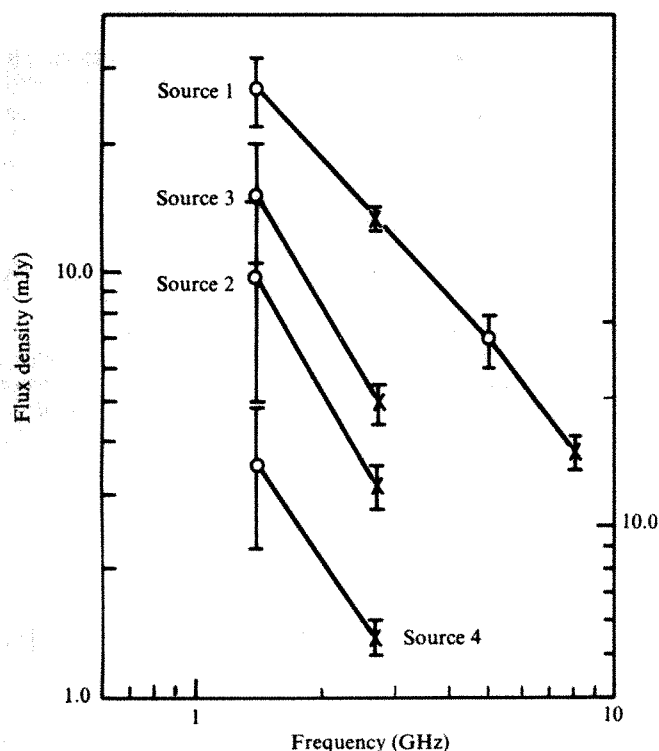


Fig. 2 Spectra of sources in Stephan's Quintet area. \times , present observations; \circ , WSRT observations².

No distinction between these two alternatives can be made with our present observations.

Table 1 shows the positions, fluxes, and spectral indices, α , for sources detected within the larger area observed. The r.m.s. errors are listed at the top of the respective columns. All fluxes have been corrected for gain of the primary beam.

In Fig. 2 we show the spectra of the three resolved sources seen within the quintet and the fourth source detected about 5' south preceding NGC7319. The 4.995 GHz flux value for source 1 is from preliminary observations by Allen (unpublished). In the case of source 4, the 1.415 GHz peak flux was read from a full field map provided by Allen (unpublished).

The most striking feature of the spectra is their steepness. Indeed, with the exception of source 1 (which falls on NGC7319), all three indices exceed the 1.3 limit observed for most extragalactic sources⁹. It is tempting to speculate that the extreme steepness of these spectra together with the extended forms of the sources may indicate that they are remnants of an earlier ejection, at least for the sources in the arc, and that the source centred on NGC7319 is of a later epoch. It should be pointed out, however, that the extreme steepness of sources 2 and 3

would be somewhat reduced if some flux were present due to an extended component that we failed to detect.

An interesting and less speculative conclusion results from the comparison of single dish observations of the area with the present high resolution measurements. Two of us⁶ have noted the probable existence of a ridge of radiation in the area of Stephan's Quintet at low frequencies. Since the present interferometric observations are insensitive to extended sources, certainly beyond about 3 arc min in extent, any excess found in a single dish measurement over the sum of the small sources falling within its beam would confirm the existence of such a ridge. Such single dish measurements within the spectral range shown in Fig. 2 (eliminating the need for extrapolation) have been made by Arp¹⁰, at a frequency of 2.295 GHz. Interpolation of the fluxes for the four sources, all falling within the beam of Arp's measurements gives a value of 34 ± 2 mJy. A similar interpolation using the whole extended arc of WSRT gives a value of 40 mJy. Arp's single dish measurement gives a value of 90 ± 10 mJy, well over twice the value expected from either of the above interpolations. We thus conclude that the evidence for a structure of ≥ 3 arc min in extent suspected at low frequencies is strengthened by the present results which strongly support the existence of a broad component at the higher frequencies.

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Seismographic observation at the bottom of the Central Basin Fault of the Philippine Sea

THE Central Basin Fault of the Philippine Sea is proposed as an extinct mid-oceanic ridge^{1,2} and seems to be a key to the development of the floor of the Philippine Sea. Though the fault seems to be aseismic on the basis of land networks, it is interesting to know whether microearthquakes occur in the vicinity of the fault. We put a sensitive ocean-bottom seismo-

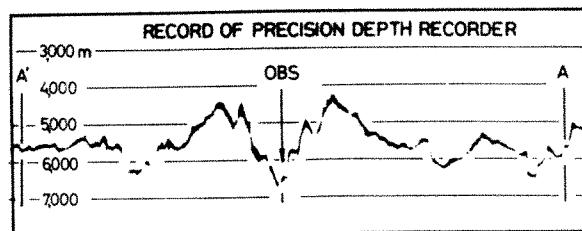


Fig. 1 Locations of observation sites of ocean-bottom seismographs. Bathymetric map (depths in fathoms) is reproduced from HO 1301 publication. Intersection lower left is a record of ship-borne depth recorder of RV Hakuho-maru. Position of A: $15^{\circ}06'N$ ($15.10^{\circ}N$), $129^{\circ}54'E$ (129.91°). Position of A': $16^{\circ}43'N$ ($16.71^{\circ}N$), $131^{\circ}15'E$ (131.24°). Direction from A to A': 39° (perpendicular to the fault); distance from A to A': 233 km.

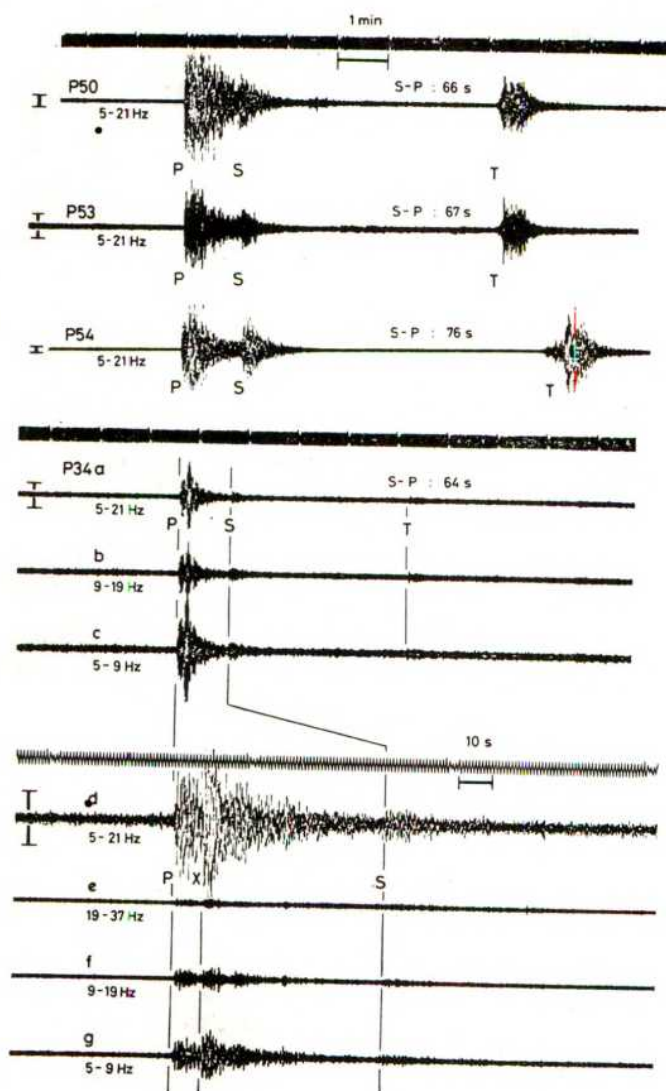


Fig. 2 Some earthquakes recorded by OBS. Number of events, frequency band of band-pass filters which were used in reproduction of magnetic tapes, S-P time as indicated. T phase travel time data support the view that the velocity of T is 1.5 km s^{-1} . Equivalent ground velocity amplitudes of $15 \times 10^{-6} \text{ cm s}^{-1}$ are also shown by bars on each trace. The X-phase is discussed at the end of the paper.

graph (OBS) in the median valley of the ridge (the width of which is only a few kilometres) and from its recordings have been able to deduce the existence of microearthquakes. It has also been possible to estimate P and S velocities for the top of the mantle and a Q structure for the upper mantle.

Seismographs have been developed over the past few years^{3,4} for seismographic survey in the vicinity of deep sea trenches of the western Pacific⁵ and for long-range explosion experiments in ocean basins⁶. The instruments were intended to record low level seismic signals and were designed to be buried in the ocean sediment to avoid the noise generated by ocean-bottom water currents.

The position of the bottom seismography (by satellite navigation) was 16.03°N , 130.72°E (Fig. 1). The depth was 6,500 m and the bottom was covered by sediment. The recording period was about 63 h, from 0930 GMT on September 16 to 0030 GMT on September 19, 1973. More than 120 earthquakes were recorded, none in the vicinity of the observation site. Considering the sensitivity of the seismograph, no earthquake whose magnitude was more than -3, 0 or 1 occurred within 20, 100 or 250 km of the observation site. All the earthquakes observed, except one, took place beyond the edges of the Philippine Sea

plate as they have S-P times of more than 64 s. The exception was an earthquake with an S-P time of 25 s, and a magnitude of about 2.

The numbers of earthquakes recorded, 2 h^{-1} , and the distribution of S-P times indicate high sensitivity of the bottom seismograph (the distances from epicentres being more than 700 km) and a high level of seismicity along the Philippine and Ryukyu trenches.

Travel times of an earthquake near Taiwan, located by the WWSSN network (0709 GMT on September 18, 24.9°N , 122.0°E , $h = 86 \text{ km}$, $M = 4.9$), show that the mean V_p value is 7.9 km s^{-1} and mean V_s value is 4.6 km s^{-1} , when the epicentral distance is 1,340 km. The mean V_p value is considerably lower than the 8.2 or 8.3 obtained in the western Pacific Basin for similar epicentral distances (T.A., H.S., Y.T., and K. Kobayashi, unpublished). The S-P velocity is 11 km s^{-1} . The value of V_p indicates that the upper mantle velocity beneath the Philippine Sea is considerably smaller than that of normal ocean basins.

The seismograms we recorded have strangely small amplitudes of S phrases (Fig. 2). Although only a vertical seismometer (natural frequency 3 Hz) was used in this observation, the marked diminution of amplitudes of S waves contrasts markedly with seismograms of similar S-P times recorded at the other observation sites of our bottom seismographs. It is probable that shear waves are greatly absorbed in the upper mantle beneath the Philippine Sea; this perhaps results from partial melting not unconnected with high heat flow data (Y. Kono, and Y. Tomoda, personal communication).

Spectral analyses of the initial part of P waves were performed. If we assume the source spectra of earthquakes, the mean attenuation factor \bar{Q} along the wave path can be calculated from the slopes of spectral curves (Fig. 3). Differences in the assumed source spectra probably result in a trifling difference in the estimation of \bar{Q} because magnitudes of earthquakes do not differ by much in this study. The estimated \bar{Q} values are shown

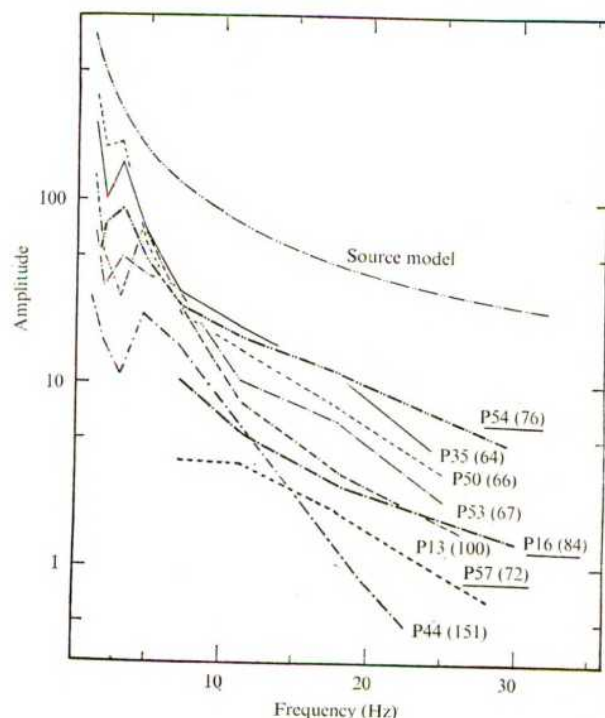


Fig. 3 Some examples of frequency spectra of first part (initial 3 s) of P waves. Number of event and S-P times (in parentheses) are shown at each curve. Note that high frequency components of earthquakes with S-P times of 70-80 s are more abundant than those of 64-67 s. One of Aki's source models⁸ is also shown.

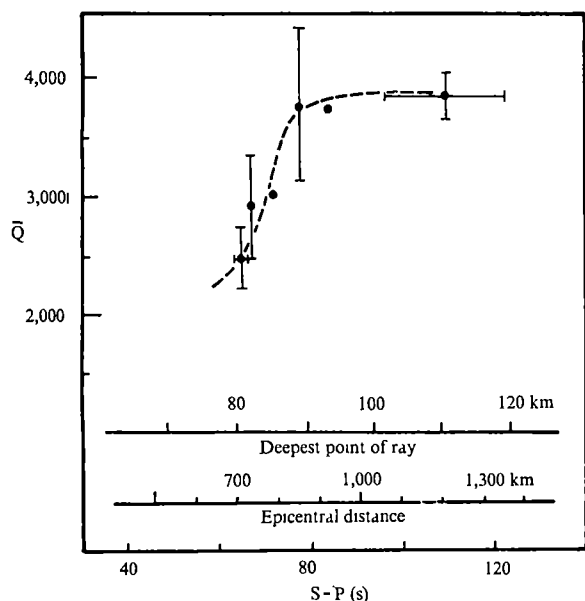


Fig. 4 Distribution of \bar{Q} values. In the computation, the ω^{-1} model for the source of these frequencies (or model B of Aki⁵) was used. Although other source models will produce different absolute values of \bar{Q} , an abrupt increase of \bar{Q} value at the same range and the same depth is observed.

in Fig. 4, illustrating an abrupt increase in \bar{Q} value at a distance of about 800 km. This suggests that a high \bar{Q} layer exists. The depth to the boundary of this steep increase of \bar{Q} value cannot be accurately estimated because the velocity structure is not known, nevertheless it is about 80 km if we assume the Japanese Meteorological Agency travel timetable, which fits well in and around Japan. Although \bar{Q} values of about 2,000 are higher than those of island arcs such as Honshu, the values are still considerably lower than those of ocean basins obtained from other OBS observations.

Most of the recorded seismograms have clear secondary arrivals at 8–9 s after onsets of P waves (see the X-phase on Fig. 2).

This peculiar phenomenon can be explained if the secondary waves are P to S conversions at the boundary at which \bar{Q} value is abruptly increasing. The highly attenuative zone underneath the western part of the Philippine Sea is probably bottomed by a high \bar{Q} layer at a depth of about 80 km. This structure is quite different from that of the north-west Pacific Basin (T A, H S, Y T, K Kobayashi, S Murauchi, N Den, and T Asanuma, unpublished) and could be peculiar to extinct mid-oceanic ridges.

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Bounds on the P velocity for the whole Moon

THE hedgehog algorithm suggests that either some Apollo seismic travel time data are in error (some layers dip) or a high velocity lid exists at a depth of 60 km in the Moon. The velocity depth curves for the Moon derived from the Apollo seismic data^{1,2} are probably not representative of the Moon as a whole and lateral inhomogeneity in the near surface layers can affect the generality of measurements of arrival time and amplitude.

We are particularly cautious about amplitude data, as plausible lateral variation in the near surface layers of the Moon can result in substantial errors in the estimates of ray

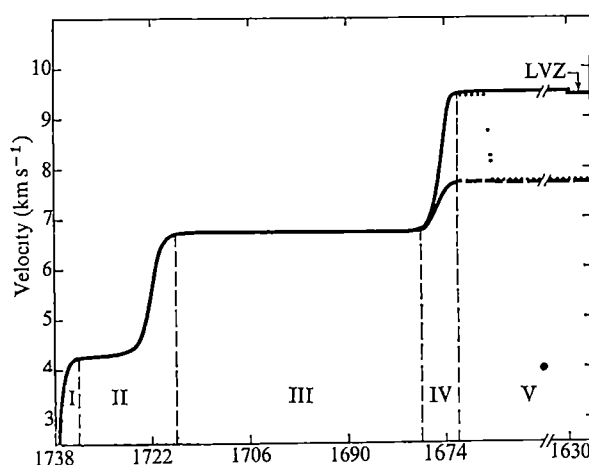


Fig. 1 Initial models for a hedgehog search of lunar structures ———, Class A, — — —, class B, — · — · —, class C

parameter based on a radially homogenous Moon. Since amplitudes depend on derivatives of ray parameters we suggest that the use of amplitude data to elucidate lunar structure is premature.

The first few kilometres of the Taurus-Litrow region of the Moon have a step-wise velocity depth structure compatible with a series of lava flows³. Travel time differences of up to 1 s can arise if the thickness of any lava layer

Table 1 P wave travel times used in the inversion

Delta (degrees)	Travel time (s)	Weights
0.287	5.57	4.0
2.2	17.8	1.75
3.07	22.0	1.75
3.76	25.0	2.0
4.45	28.6	2.0
5.18	32.0	2.0
5.67	35.7	2.0
6.07	26.6	2.0
11.2	57.0	2.0
11.8	55.0	2.0
—	61.0	2.0
28.0	123.1	2.0
34.0	155.1*	2.0
	151.0*	1.5

*Only one of these two arrivals was used, in class B models, which one is used affects the half space velocity acceptance region by about 0.5 km s⁻¹.

is doubled or halved. As such variations would introduce errors in any analysis which considers the Moon to be laterally homogeneous, we add them to those observational errors which we have estimated as 0.5 s from a few sample seismograms¹. We considered variable accuracy by assigning weights based on discussions in the literature^{1,2}.

Solution of the inverse problem for such a set of travel time observations is well suited to the hedgehog algorithm⁴. This algorithm can deal with amplitude data and with travel times. Until the effect of intensive scattering is understood and synthetic seismograms can be made to fit substantial portions of lunar seismograms, however, we hesitate to use such data.

We used three models (Fig. 1) as a starting point in our search for a range of models which would fit the data within the accuracy of the experiment. The criteria for fit are: (1) the root mean square error of fit shall be less than σ , (2) no more than j time observations shall differ from computed times by k times the weight W_i of the i th phase.

Table 2 P wave travel times*

Delta (degrees)	Travel time (s)	Weights
11	10.5	2.0
15	14.0	2.0
19	17.5	2.0
518	35.5	2.0
112	54.0	2.0

*Note the considerable uncertainty in these points. They were read from a graph of travel times prepared by Toksoz *et al.*¹

We used various values for σ , j and k , and coarse searches in which positions were changed by increments of 10 km and velocities by increments of 1 km s⁻¹, together with fine searches with increments of half this size. We could not find any acceptable models of class A (Fig. 1) with any reasonable set of σ , j , k values. The data for the triplication (from either Table 1 or 2) suggest a half space velocity of 9.5 km s⁻¹ while the travel times near 30° suggest a velocity of 7.75 km s⁻¹.

Class B models all have a poorly resolvable low velocity zone (LVZ) at a depth of about 100 km. With an LVZ

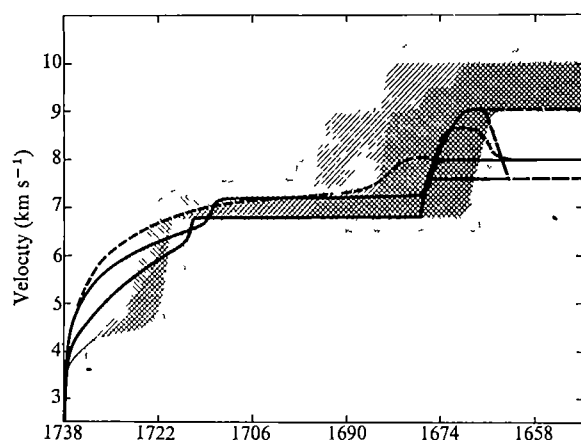


Fig. 2 Various solutions for the lunar velocity depth curve. The lines are published^{1,2} solutions using both amplitude and travel time data. The cross-hatched area is the region delineated by a fine hedgehog search. The lined area plus the cross-hatched area is derived from the coarse search. The stippled area illustrates the uncertainty caused by discrete steps taken in the hedgehog search.

Table 3 P wave travel time data at large deltas

	Delta (km)*	Travel time (s)	Weight
Meteoroid impact of day 134, 1972	967	131.5	1.0
	1,026	138.5	1.0
Apollo 16 SIVB	1,099	147.0	0.4
From Table 1	850	123.0	2.0
	1,032	151.0	2.0
		155.1	2.0

*30.3 km = 1° on the lunar surface

These observations depend in part on an assumed model of the Moon's mantle

located at this depth we have no direct P arrivals near 30°. Multiples (for example, PP) may be possible. If the arrivals at about 1,000 km are PP: (1) Using data in Table 1, together with the acceptance criteria ($\sigma=2.8$, $j=0$, $k=1.2$) and a coarse grained search, we found some acceptable structures (Fig. 2). (2) Using data in Tables 1 and 2 ($\sigma=6.0$, $j=0$, $k=2.4$) and a fine search we found additional acceptable structures. (3) Using data in Tables 1, 2 and 3, no acceptable solutions were found in a search based on ray theory alone. If the uncertainties (σ and k) are reduced to half the values in cases (1) and (2) there are no acceptable solutions on the basis of a fine search. If the data in Table 3 are in error by more than 10 s, solutions (1) or (2) are correct, otherwise, models A and B are inadequate. If class B models and Fig. 2 are accurate, high velocities in the first half space raise strict petrological constraints¹. Class C models are characterised by a thin lid of high velocity. Classical ray theory is not applicable in a thin layer. We expect, instead, a head wave in the lid whose low frequency components will tunnel into the low velocity zone beneath it. This tunnelled phase will be effectively low-pass filtered while the headwave is high-pass filtered.

The anomalous data at 1,000 km can be interpreted as a combination of tunnelled waves and head waves⁵. The velocity at the top of the lid is between 8.0 and 9.5 km s⁻¹, below which a constant velocity (7.25–7.75 km s⁻¹) half space exists. A thin high density garnet layer would be stable at this depth in the lunar interior⁶.

With the exception of the first 8 km of the Moon and the mantle velocity, the range of our solution spans published solutions which have used both travel times and amplitudes. We used a one parameter model for the near surface structure. Had we used a more complex model our solution would also have spanned the published solutions in the near surface layers. We feel that detail in the near surface layer is an unjustified complication. The discrepancy below 1,665 km is resolved if head waves and tunnelled waves exist in a high velocity lid.

We thank G. V. Latham for making available the data in Table 1, and M. N. Toksoz for the results in Table 2.

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Short period teleseismic S waves

SHORT range observations of short period S waves are useful in determining the distribution of zones of high and low anelastic attenuation in the vicinity of island arcs¹⁻³. We show here that S waves are also recorded at some stations at teleseismic ranges and that these recordings can be used to study anelastic attenuation.

S waves are useful because they are more sensitive to changes in anelasticity than P waves. The attenuation of body waves at angular frequency ω is given by $\exp(-\omega t^*/2)$ where $t^* = T/Q_{av}$, T is the travel time and Q_{av} the average quality factor Q , for the raypath and the particular wave type. As S wave travel times are about $\sqrt{3}$ of the travel times of P waves, and Q_p is about $4/9$ Q_s (making the usual assumptions that anelasticity affects only the shear modulus of rocks^{4,5} and that Poisson's ratio is around 0.25), t^* for P waves (t_p^*) is roughly a quarter of t^* for S waves (t_s^*). Thus, for an S wave travel

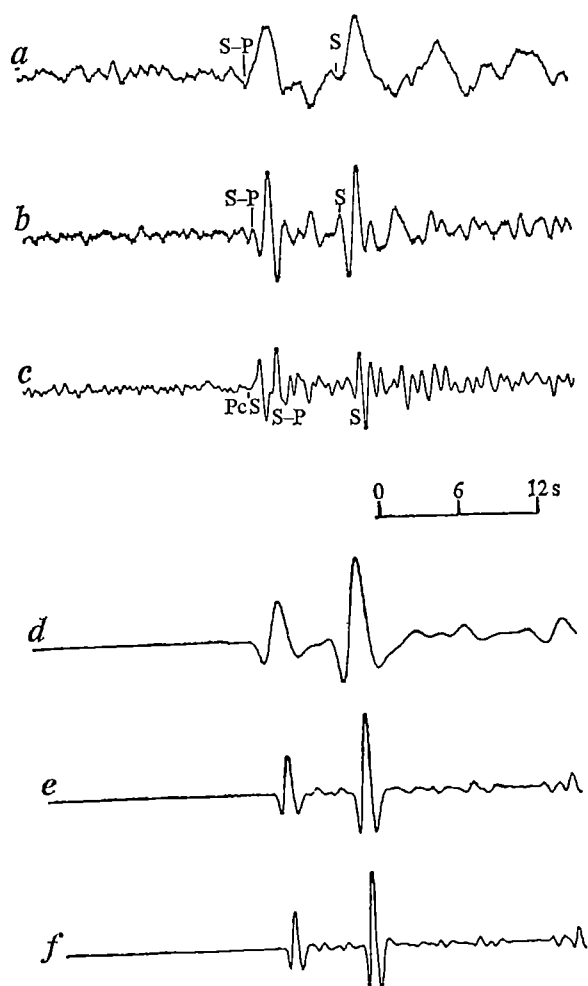


Fig. 1 Observed (a-c) and modelled (d-f) S wave seismograms showing double arrivals. The later arrivals on the observed seismograms are interpreted as true S waves and the preceding arrivals as S to P conversions at the Moho beneath the receiver. The modelled seismograms include no effects of layering in the source region, all the arrivals are generated from a single S wave arrival at the base of the receiver crust. Note that the PcS wave for earthquakes at the depth and distance illustrated in c (P wave reflected from the core as an S wave) arrives just before the S-P arrival. a November 2, 1972, Loyalty Islands, origin time 19 h 55 min 22.1 s, $m_b = 6.3$, $\Delta = 32.40^\circ$, $h = 32$ km. b October 26, 1972, New Hebrides Islands, origin time 22 h 48 min 34.4 s, $m_b = 5.4$, $\Delta = 31.9^\circ$, $h = 157$ km. c March 9, 1971, south of the Fiji Islands, origin time 08 h 11 min 52.8 s, $m_b = 5.0$, $\Delta = 42.4^\circ$, $h = 511$ km. d, $t_p^* = 3.5$, e, $t_p^* = 1.3$, f, $t_p^* = 0.7$.

Table 1 Crustal model for receiver crust

	P wave velocity (km s ⁻¹)	S wave velocity (km s ⁻¹)	Density (g cm ⁻³)	Thickness (km)
1st layer	5.6	3.2	2.8	12.0
2nd layer	5.9	3.4	2.8	12.0
3rd layer	6.2	3.6	3.2	14.0
4th layer	8.3	4.8	3.4	∞

time of 1,000 s an increase in anelasticity that produces an increase in Q^{-1}_p of 0.001 gives, at 1 Hz, 10 times more attenuation of S waves than of P waves. Short period S waves are difficult to detect at teleseismic distances because for many paths through the Earth t_p^* is apparently about 1.0 s or greater^{6,7} so that t_s^* will be 4.0 s or more. Thus (at 1 Hz), S waves will be about 10,000 times smaller than P waves, at the receiver, other things being equal.

Values of t_p^* in the range 0.2-0.5 s have, however, been reported^{8,9} on some paths, suggesting that differences between the attenuation of P and S waves for these paths should be in the range 10-100. Short period S waves should, therefore, be detectable at long ranges. A search of recordings from array stations Yellowknife, Canada (YKA) and Warramunga, Australia (WRA) shows that both stations do sometimes record short period S waves from earthquakes with (US Coast and Geodetic Survey) body wave magnitudes greater than 4.8. The largest S waves relative to P waves are from deep earthquakes. As most attenuation is thought to occur in the asthenosphere at depths in the Earth of 100-300 km, and the

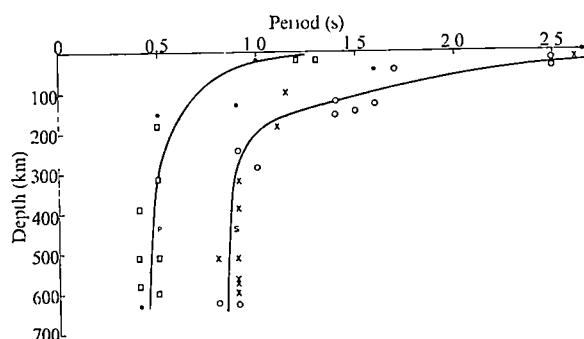


Fig. 2 Dominant period of direct P and S waves observed at WRA as a function of the depth of the source, combined data from Tonga-Fiji (\square, \times) and New Hebrides (\bullet, \circ) regions.

asthenosphere is thought to thin under shields, then observations of large S waves from deep earthquakes at stations such as YKA and WRA, which are situated on shields are just what is expected (compare Fig. 1a and c).

The largest number of S waves that we have obtained are from WRA for the Tonga-Fiji and New Hebrides regions, and as these are the regions where Q has been most extensively studied at short ranges, we shall concentrate on these observations.

Figure 1 (a, b and c) shows WRA recordings of SV waves (the array contains only vertical component seismometers) from three earthquakes. All the examples show two distinct arrivals separated by 6 s, both have apparent surface velocities close to those predicted by S wave travel-time tables. The second of the two pulses seems to be a true S wave, the first is interpreted as a P arrival generated by S to P conversions at the base of the crust at WRA. Explanations such as double sources can be ruled out because the P waves show no similar double arrivals, and the possibility that the second pulse is a depth phase can be discarded because the double pulse is generated by earthquakes at all depths with a constant separation of about 6 s.

The existence of P precursors to SV have been suspected for many years Jeffreys¹⁰, for example, proposed them as an explanation of the difficulties that occurred in reconciling P and S travel times, for it seemed that S waves from many earthquakes arrived earlier than predicted

The double pulse can be modelled theoretically (Fig 1d, e and f) using the method described by Hudson^{11,12}; the WRA crustal structure used (Table 1) is based on a model¹³ for the Canadian Shield Kanesewich¹⁴ *et al*, have made similar theoretical calculations and discussed the implications of precursors to S waves, and Báth and Stefánsson¹⁵ have observed precursors to S waves at teleseismic distances on long period instruments. The examples shown here seem however, to be the first short period observations of such precursors at these distances, although Smith¹⁶ has observed similar effects from local earthquakes

To ascertain the effects of attenuation we measured the dominant period of the P and S waves as a function of the depth of focus (see Fig 2 and ref 2). As there are no obvious differences between the data from Tonga-Fiji and those from the New Hebrides they are both shown on the same figure. The results show an increase in the period of both the P and the S waves with decreasing depth of focus for depths shallower than 250 km but, as expected, the increase in period is much more pronounced for S waves than for P waves. Assuming that these differences in period can all be attributed to the effects of anelastic attenuation it is possible to deduce a rough *Q* structure for the upper mantle in the Tonga-Fiji and New Hebrides regions (Table 2). The structure was obtained by modelling P and S recordings for a range of values of t^* and measuring the predominant period of the resulting seismograms. Using this relationship between t^* and period, we estimated the value of t^* for paths to WRA for each depth of focus (using the smoothed curves drawn through the data, Fig 2). The upper mantle was then divided up into a series of layers in which *Q* is assumed constant, and the *Q* structure was derived using the time spent in each layer by rays travelling to WRA for each depth of focus.

The result shows very low *Q* values at depths shallower than about 200 km in both the New Hebrides and Tonga-Fiji regions. The low *Q* in the Tonga-Fiji region seems to coincide with the zone of "extremely low *Q*"² beneath the Lau Basin west of the Tongan Ridge, although we shall have to use ray tracing to work out the details of structure. The similar zone of extremely low *Q* lying to the south and west of the New Hebrides region does not seem to have been reported previously.

Table 2 Approximate *Q* structure for the upper mantle

Depth (km)	Q^{-1}_a	Q_a	Q^{-1}_b	Q_b
0-100	0.0250	40	0.0500	20
100-225	0.0110	90	0.0220	45
225-600	0.0010	1,000	0.0033	300
600	0.0004	2,500	0.0008	1,250

In deriving the *Q* structure we have made the assumption that any source effects on the observed period can be neglected. This is probably an oversimplification, it may be that for shallow sources the pulses radiated are of longer duration and, therefore, the ratio between high frequency energy and low frequency energy is lower than for deeper sources. We have, however, found it impossible to model seismograms showing S waves with a period of over 2 s by increasing the source size while obtaining a good fit to the P phase at the same time. For example, PKiKP is visible for the Loyalty Islands earthquake (although not illustrated in Fig 1a) as a single wavelet with a period of 0.8 s, and the S phase for this earthquake has

a period of 2.5 s. With the source model used here these differences in period cannot be shown as an effect of source rather than of attenuation.

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Possible mechanism of continental breakup in the North Atlantic

New observations from the Skaergård area have provided evidence relating to the mechanism of continental breakup in the North Atlantic. The East Greenland coastal flexure and its associated dyke swarm¹ has become a classic of this type of structure and has been frequently compared with similar structures around the world, notably the Lebombo monocline of south-east Africa^{2,3}. No new data have appeared in print, however, since the original description by Wager and Deer¹. I have had the opportunity to examine the structures in the Skaergård area (68°N, 32°W), and report here my observations on the mechanism of flexuring and dyke intrusion, which is believed to be very similar to structures described from areas of present spreading, such as Afar⁴.

In the original description Wager and Deer emphasised that the coast parallel dykes were intruded radially to the flexuring from a centre below surface, caused by tension resulting from the bending of a thick succession of lavas (Fig 1a). Closer examination of a 500-m profile at nearly right angles to the coast at Haengefjeldet, south of Skaergård (Fig 2), has shown that the continuous decrease in dip on approaching the coast, described by Wager and Deer is a severe over-simplification. Most of the dykes in the swarm have average dips of 90-70° N and strike 90-100°, an orientation which seems to be rather constant from the coast and several kilometres inland. All dykes have very irregular dips and several are found which have dips varying from northerly to southerly directions. Examination of the dyke chulls in the walls showed that dilatation is the normal intrusive mechanism and that the host rock in

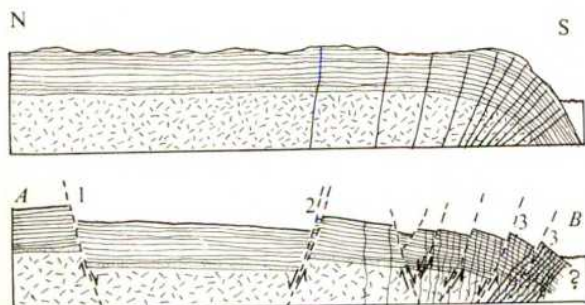


Fig. 1 Sketches of the profile A-B (Fig. 2) showing (upper) the model proposed by Wager and Deer¹ compared with (lower) that proposed in this paper. The older generation of dykes is shown in heavy black, the younger as the thinner, approximately vertical dykes. Numbered faults correspond to those shown in Fig. 2.

several places is an older dyke swarm of thick, black, coarse-grained dykes.

This older dyke swarm which is also parallel to the coast, is very uniform in the field and in hand specimen and has low dips of $50-40^\circ$ N. It cuts the lavas, agglomerates and tuffs of the lower basalt series³, which are orientated $100/45-60^\circ$ S and so is perpendicular to these. Both lavas and older dykes are cut by numerous small shear zones and faults, now filled with carbonate and zeolites. The vesicles of the lavas are also completely filled. The mineralogy of the older dykes and the lavas has suffered from this altera-

tion such that olivine always is replaced by chlorite, serpentine, carbonate, and so on, while plagioclase is less altered and pyroxene is always fresh. The large amount of pseudomorphed olivine both in the groundmass and as phenocrysts, suggests that the older dykes were very basic olivine basalts. Chemical work on this subject is in progress and will be presented elsewhere.

In contrast, the younger main swarm with grey, green, brown and red weathering colours shows no signs of shearing and faulting and is much less altered. As suggested by the weathering colours the younger swarm is characterised by a wide spectrum of different rock types, including both aphyric and plagioclase-aphyric dolerites together with a range of intermediate to acid types. The distribution of dykes with a concentration near the coast described by Wager and Deer¹ and confirmed by my own observations, was regarded by them as being the consequence of the maximum bending of the coastal flexure in this area (Fig. 1a). It now seems, however, that the high intensity of dykes near the coast is caused by the presence of both dyke generations within 1 km from the coast, whereas only the younger generation persists further inland, at least up to 5 km, with little or no decrease in intensity.

These observations clearly show that the younger dykes were not intruded perpendicular to the lavas and that they were intruded after the tilting of the lavas. The older dykes, which, in common with the lavas, are sheared and altered, must have been formed before the flexuring, and thus neither of the generations can have formed as the result of the process described by Wager and Deer.

The faulting and shearing described in the profile at Haengefjeldet has been found in the whole of the Skaergård area, and the profile A-B (Fig. 2) will now be described in some detail. In the description of the Skaergård area, Wager and Deer⁶ noted the Sorte Kap fault 60 km inland as a normal fault with downthrow to the south and a dip-slip of at least 800 m. North of this fault the lavas dip slightly north and to the south dips are small but southerly (Fig. 1b). At the lake in Sødalen, Mikis Fjord, a normal fault was located running E-W and dipping north with a downthrow to the north and a dip-slip of several hundred metres, shown by the fact that the underlying sediments are relatively up-thrown and exposed to the south. North of this fault the lavas dip 10° S while to the south of it the dips have increased to $20-22^\circ$ S.

Although minor faults occur, dips of the lavas are more or less constant as far as 5 km from the coast along the Denmark Strait. Because of the intensity of the younger generation of dykes in the dyke-swarm along the coast, not disturbed by faults, outcrops of the lava succession are difficult to correlate, but within the last 5 km to the coast, dips change from 22° S to $45-60^\circ$ S. It is believed that this change occurs principally at two or more major shear zones, which have been seen in the outer Mikis Fjord and at Haengefjeldet.

Shear zones have been found to be very common in the coastal region but at most localities displacements are very difficult to evaluate, because of the lack of marker horizons. For example six shear zones or faults cut the Skaergård peninsula, but in only one of these could the displacement be confirmed by a dyke intersection.

These relationships in Mikis Fjord area are very similar to those described by Mohr⁴ from the western escarpment of the Afar triangle in Ethiopia. The model proposed for the East Greenland coastal flexure, is that of a similar large scale fault pattern with marginal normal faults dipping S (such as the Sorte Kap fault) and a large number of smaller northerly dipping faults near the coast with downthrow to the north, causing a rotation of fault blocks and the dip variations in the lavas.

This interpretation corresponds to a 'half' graben structure, which is consistent with the plate-tectonic origin of

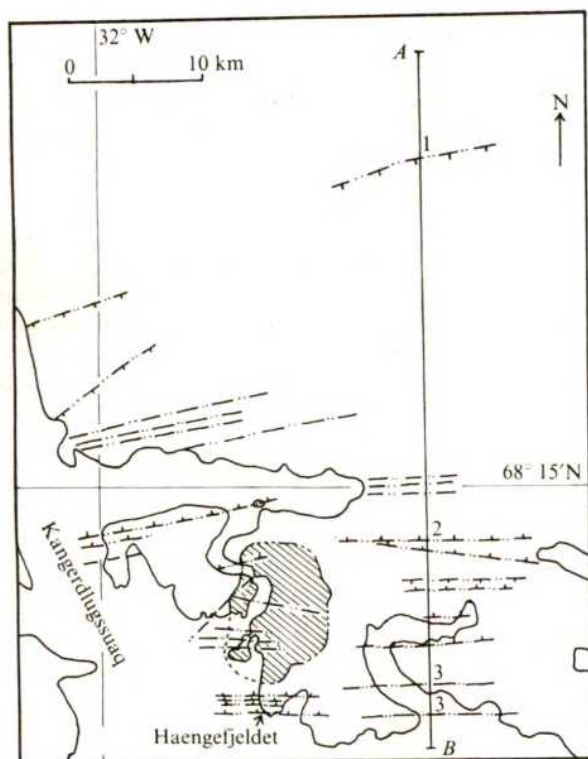


Fig. 2 Simplified map of the Skaergård area, East Greenland. The Skaergård intrusion (shaded) and faults with dip directions are shown. Faults without dip signs are shear zones where the faultplane is more or less vertical and relative displacement has not been determined. 1, Sorte Kap fault; 2, Sødalen fault; 3, Mikis Fjord major shear zones (Fig. 1b).

the lower Tertiary geology of this area. Thus, Brooks⁷ described the Kangerdlugssuaq areas as the site of the Icelandic mantle plume in Eocene times, which resulted in major updoming, rifting about a triple point and subsequent crustal separation along fractures parallel to the present coastline.

As it is assumed that both dyke-swarms and the flexuring of the coast are formed in the breaking up of the North Atlantic, the correlation between dyke intrusion and major tectonic events might be explained in the following manner. As stated earlier, both the older dykes and the lavas are flexured and therefore must be older than the collapse of the coast marked by the faulting and shearing. Thus these dykes may well be correlated with the prespreading updoming described by Wager and Deer⁸ and Brooks⁷.

The younger dykes, which are not sheared and faulted, were probably formed after the initial fracturing event and could be correlated with the active spreading and new crust formation, which opened up the North Atlantic.

Finally, the recognition of intense faulting in the area indicates that extreme caution must be exercised when employing the structural heights in the Skaergård intrusion as estimated by Wager and Brown⁸. Tectonic disturbances of the intrusion would explain some of the irregularities of the Skaergård contact, which can be seen on aerial photographs and the map of Wager and Brown⁸. Such faulting would also be expected to have a profound effect on the magnitude of the hidden layered series in the intrusion, whose size has recently been questioned by geophysical⁹ and geochemical¹⁰ investigations and on estimation of the thickness of the basalt succession in the area.

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Interaction between coherent light waves and free electrons with a reflection grating

A NEW effect is proposed for obtaining effective interaction between coherent light waves and free electrons. Potential applications of the effect are optical electron accelerators (linac)^{1,2} and optical amplifiers³.

Smith and Purcell demonstrated⁴ that light is emitted when a high voltage electron beam moves parallel and close to a metallic optical diffraction grating in a direction perpendicular to the grating rulings (Fig. 1a). The radiation has been explained physically in terms of the oscillations that must be executed by the charge induced on the grating surface by an electron in the beam. The dispersion relationship is

$$\cos \theta_n = (c/v) - (n\lambda/d) \quad (1)$$

with $n = 1, 2, \dots$, θ_n the angle that the direction of propagation

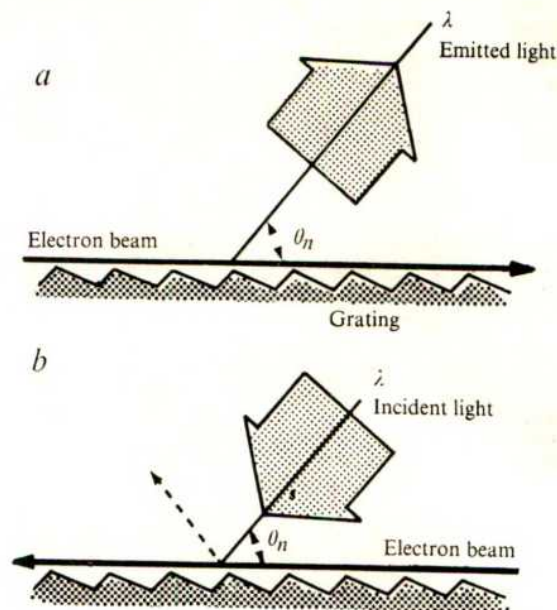


Fig. 1 a, Schematics of the Smith-Purcell radiation; b, 'inverse Smith-Purcell effect'.

makes with that of the electron beam, d the grating constant, v the velocity of the beam, and λ the free space wavelength of the light.

Here we consider the 'inverse Smith-Purcell effect' to obtain extended interaction of an electron beam moving along the grating surface with light incident on the surface. A simple Huygens analysis shows that when the directions of the incident light wave and the electron beam are opposite to those in the case of Fig. 1a and satisfy the condition of equation (1), the beam interacts synchronously with the wave; that is, an electron sees the same phase of the wave with the pitch of d (Fig. 1b). When the electron beam is opposite in direction to the wave on the grating surface, the synchronous condition becomes

$$\cos \theta_m = (m\lambda/d) - (c/v) \quad (2)$$

where $m = 1, 2, \dots$

When the synchronous condition is satisfied, an effective interaction must occur between the electron beam and the light wave in the same manner as the spatial harmonic interaction occurring in microwave travelling-wave tubes, leading to

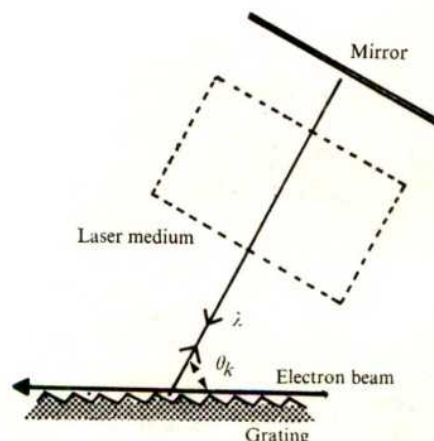


Fig. 2 The Smith-Purcell and 'inverse Smith-Purcell' arrangements with a Fabry-Perot resonator.

electron acceleration or deceleration according to the relative velocities of the electron and the phase of the light wave in the interaction region. If the electron suffers net acceleration over a period of time, then electromagnetic field energy is converted into electron kinetic energy, resulting in an electron accelerator. Optical (laser) accelerators will have the unique property of providing high acceleration gradients because of the intense fields that can be obtained. An acceleration of the order of 1 GeV m^{-1} may be anticipated³. In addition, the electrons will be bunched to a dimension which is short compared to the light wavelength, giving current pulses with a time duration of the order of $\sim 10^{-15} \text{ s}$. These pulses could be used to study ultra-fast relaxation processes^{1,3}.

On the other hand, when the electrons are faster than the phase velocity of the light wave and suffer a net deceleration, light amplification may be obtained.

If the incident angle θ_k of the light satisfies the following condition,

$$\cos \theta_k = k\lambda/2d \quad (3)$$

where $k = 1, 2, \dots$, the diffracted ray direction is the same as the incident one. In this case we can adopt the configuration shown in Fig. 2. A Fabry-Perot resonator is formed with the grating and a mirror⁵. Using this configuration as a laser cavity, high intensity electric field in the laser cavity can be used to accelerate the electrons. This configuration involves the one proposed by Takeda and Matsui² as the special case of $\theta_k = \pi/2$.

The same configuration can be used to obtain 'stimulated Smith-Purcell radiation', that is, the Smith-Purcell radiation with internal feedback by the Fabry-Perot resonator. We have observed⁶ the millimetre and submillimetre wave oscillation in an electron tube called the Ledatron when $\theta_k = \pi/2$.

With recent progress in the development of high power lasers in the optical and infrared regions, it should be possible to miniaturise linacs by using the 'inverse Smith-Purcell effect' proposed here.

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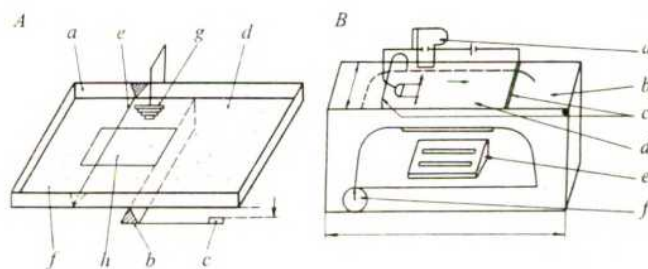


Fig. 1 Experimental apparatus. A: a, Tub; b, wedge; c, washer; d, base; e, trip-wire; f, fine sand; g, camera; h, section. B: a, Camera; b, small channel; c, electrodes; d, glass plate; e, stroboscope; f, pump.

Marked boundary layers exhibit a clearly defined reproducible pattern of streamwise streaks in their sublayers¹⁻³ and regions with higher colour concentrations are identical to those areas where liquid flow is retarded.

Observations by Fales⁴ allowed this flow structure to be explained by means of a longitudinal eddy model. As the question of this eddy structure, or its alteration in the existing models is of decisive importance^{2,5,6} in explaining the Toms effect we endeavoured to explain this problem by observation.



Fig. 2 Sand pattern experiments. a, With tap water; b, with 50 p.p.m. polyacrylamide solution.

We investigated the structures using fine sand to make the flow visible, though in fact it is not the flow itself which is observed, but the trace it leaves on the bed. As a second step we investigated the sublayer by means of the hydrogen bubbles visualisation technique.

Fine quartz sand settles evenly on the bottom of the experimental tank, and when the liquid is set in motion, the individual grains can be photographed easily, particularly when a smooth surface is used, as in this case. When we investigated integral structures, we used continuous illumination; short term movements were followed under stroboscopic lighting.

As a test device (Fig. 1a) we chose a shallow, rectangular sheetmetal trough with a smooth, black plastic foil attached to the bottom. The trough could be tipped steeply about a pivot by means of exchangeable washers, so that we could regulate the velocity of flow as a result of the tipping motion. The flow itself was also brought to a turbulent state with a trip-wire and made visible by means of fine sand. We recorded the movement of the quartz grains with a camera mounted perpendicular to the water surface. The second test was carried out in a small closed-circuit channel (Fig. 1b). The camera, or film unit, stood perpendicular to the two-dimensional flow, which was illuminated from below by a stroboscope through a glass plate.

Alteration of structures of sublayer flow in dilute polymer solutions

THE Toms effect may be assumed to be based on a phenomenon which occurs in the immediate wall layer. Models proposed to explain the effect are, therefore, mostly aimed either at producing an interaction of the molecules with the flow structure (that is, the eddies), or at attributing new rheological properties to the liquid on the basis of the molecules released, which should lead to new forms of flow.

The anode was a stretched wire 8 cm long, placed transverse to the flow and as close to the wall as possible in order to produce the bubbles in the sublayer. The cathode was used as a spillway in order to set the water depth to approximately 2 cm. The mean velocity of flow was 10 cm s^{-1} , corresponding to a Reynolds number of 2,000. As the sand tests were performed at the same Reynolds number, it was possible to compare the two results. The greatest problem, however, was the 100 p.p.m. MEYP-01 polyacrylamide solution used, because the hydrogen bubbles contributed considerably towards the destruction of the molecules and the test liquid had to be replaced with freshly prepared solution relatively quickly. For quantitative tests, therefore, the closed circuit was dispensed with, as in this case it is only permissible to work with fresh solution, which would entail substantial experimental difficulties, particularly when high demands were made of the solution's homogeneity.

But it is not only the bubbles which can alter considerably the liquid; the molecules also contribute towards distortion of the bubble pattern as a result of pollution of the anode. We took that into account with frequent cleaning of the wires. As this resulted in a conflict between an undisturbed flow pattern and flow disturbed by the cleaning, we took care to work under optimum conditions. The flow patterns were both photographed and filmed. The film was shot with a speed of 24 or 32 frames s^{-1} .

For pure water, we obtained structures which have already been observed and described¹⁻³ (Figs 2a and 3a). Polyacrylamide solutions (50 p.p.m.) produced a considerably modified sand pattern compared with that produced by pure water, distinguished particularly by the formation of dunes (Fig. 2b). As the interaction between polymer solutions and quartz particles is still not known, it is difficult to explain this phenomenon by one of the various models.

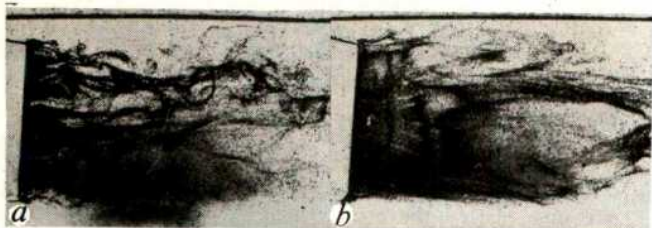


Fig. 3 Hydrogen bubble experiment. a, With tap water; b, with 100 p.p.m. polyacrylamide solution.

This observation suggests, however, that in the turbulent boundary layer of a dilute polymer solution, longitudinal eddy structures were suppressed or deflected from the direction of flow in the immediate vicinity of the bed of a smooth wall.

In the second experiment we again found a thickening of the sublayer in our tests with polymer solutions. It was clearly a transverse eddy formation (Fig. 3b) which dominated the picture.

Although the films would permit some degree of quantitative evaluation the test set-up was not designed so that figures obtained from it would be generally valid. Nevertheless, there is no doubt that the change already perceived visually may be considered as qualitatively established.

It is worth noting that Brennen⁷ observed a transverse wave structure for flow separation at spheres in the case of thin polymer solutions, which points to a similarity of the two phenomena.

Rudd⁸ provided more direct evidence of this change and demonstrated, with laser doppler measurements near the wall, a significant increase in the variation of the velocity components in the direction of flow, whereas practically no difference from pure water could be determined along the pipe axis. Exactly the opposite behaviour was found for velocity fluctuations transverse to the direction of flow; they decreased in comparison with pure water near the wall, but increased in the outer region.

This fact is immediately evident when the variations in the sublayer are derived from eddies moving past, which in the case of the polymer solutions are deflected to a greater extent from their original position in the direction of flow ($u'=0$) into the direction at right angles ($w'=0$).

Similar arguments come from local correlation measurements which, on the basis of their chronological sequence, represent a measure of the frequency of occurrence of a certain eddy structure. Thus, a considerable increase in the degree of correlation is found in the direction transverse to the flow, with a far slower decay of this correlation in the direction of flow⁹.

The appearance of a larger number of transverse eddies is an almost ideal explanation of these measurements. In the direction at right angles to the flow, the movement is strictly influenced by the eddy itself. As a result of the high stability of the eddy structures during transportation by the flow the influence lasts longer⁹.

The thickening of the sublayer also leads to an enlargement of the individual eddy diameter, so that the fundamental change of the flow structure in the sublayer varies from thin longitudinal eddies in pure water to relatively thick transverse eddies in dilute polymer solutions.

To return once more to our work with fine sand, we now understand how a dune structure occurs transverse to the direction of flow. In principle the same as the longitudinal eddy structure, but it is wider because of the thicker sublayer.

This, however, only explains the formation of dunes, not their marked wedge shape, which must be the result of a locally varying transfer process. As the dune bank formed transverse to the flow is eroded at the point where the flow in this sublayer exhibits higher Reynolds stresses, it is natural to consider the sand pattern as a dune formation which is eroded locally by shearing waves.

The pattern itself has a self-stabilising effect, as the shearing waves are channelled by the topography of the sand. The stochastic disturbances which possibly still occur locally and sporadically, and which spread out as shearing waves, are thereby fixed; this results from an interaction with the mobile bed. So no comment can be made about the local stability of the disturbances; only about the relationships between wavelengths in both directions of propagation. This relationship determines the mean direction of propagation of the disturbances and can be found from the characteristic apex angle β . Assuming that these disturbance waves appear in the wedge structure β can be measured (Fig. 2b). For our test, it averaged $14.5 \pm 1.5^\circ$.

Morrison¹⁰ *et al.* specified a disturbance wave model for sublayers and took measurements by frequency wave number spectra on turbulent flow in pipes.

Using shearing velocity and kinematic viscosity as scaling values, they found, expressed dimensionless in these magnitudes, well-defined wavelengths transverse to the flow and very weakly defined wavelengths in the direction of flow. The characteristic angle was 12° .

The sublayer is thickened substantially by the addition of polymers and the disturbances are propagated in the form of shearing waves, which do not differ greatly from those in pure water.

This alteration in structure, as can be demonstrated clearly, indicates that it would be premature to draw speculative conclusions at this stage about the Toms effect itself.

Rather, it needs to be explained why the concentration of vorticity is rotated.

As one of the most obvious explanations lies in associating this behaviour with normal stresses, this indicates an elastic effect.

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Lead monolayer lubrication in steel machining studied by Auger electron spectroscopy

ALTHOUGH free-machining steels are widely used in the manufacturing industry because of improved machining characteristics, such as prolonged tool life, the detailed mechanisms by which the additions of lead and manganese sulphide bring this about are subject to debate. We report the existence, on the swarf from leaded free-machining steel, of near monatomic layers of lead which are thought to enhance the lubrication of the swarf-tool interface.

The steel was dry machined at six cutting speeds in the range of 50–350 feet min⁻¹ (0.25–1.78 m s⁻¹) using a new M2 high-speed steel cutting tool for each speed. The cutting tool geometry conformed to the relevant International Standards Organisation recommendation. Samples of the swarf were carefully collected to avoid contamination and were then analysed by Auger electron spectroscopy (AES).

AES is an electron probe technique which gives an elemental analysis of the few uppermost atom layers at a surface. Combined with ion beam etching, AES provides element composition profiles of the surface layers with in-depth resolutions as good as 1 nm. Figure 1 shows a typical spectrum of a swarf surface (air-exposed) which had contacted the rake face of the tool, indicating a very strong concentration of lead and manganese sulphide in the surface layers of the swarf compared with the bulk. The uniform distribution of the lead over the swarf surface is illustrated in the 'Augergraph' in Fig. 2. The lead Auger electron signal has been used as a means of recording the distribution of lead; this resembles the use of X rays in the conventional electron microprobe but here refers more critically to the one-or-two atomic layers nearest the surface. Similar spectra to that in Fig. 1 were obtained from the other side of the swarf which had previously contacted the flank face of the tool.

Further information on the composition of the surface layers of the swarf was obtained by ion beam etching. Figure 3 shows that both carbon and lead were largely removed from the surface in the period of the ion bombardment, whereas manganese remained. The decrease in sulphur concentration is an artefact, attributed to oxidation of manganese sulphide induced by electron beam irradiation in the presence of residual water vapour in the Auger electron spectrometer. This was checked by moving the electron beam to a neighbouring position on the etched surface, thus restoring the sulphur signal to its original value. The ion bombardment was continued

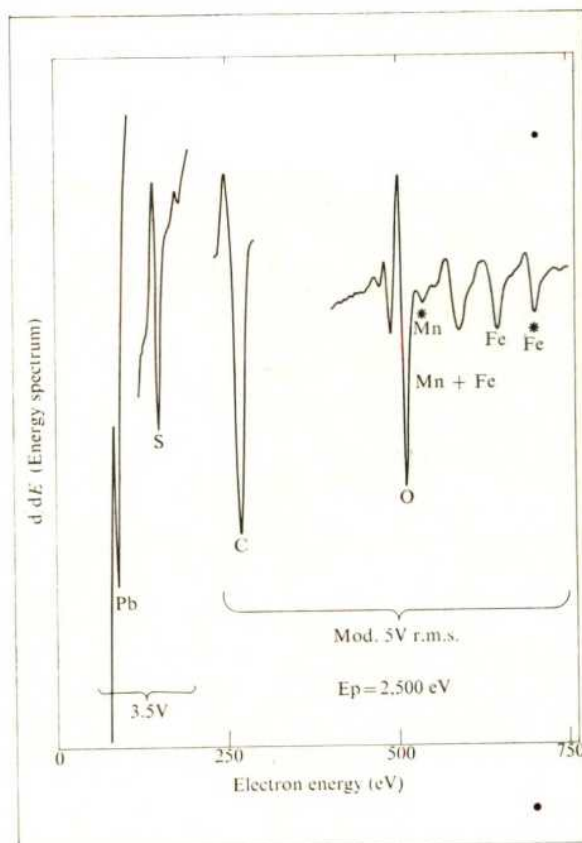


Fig. 1 Auger electron spectrum of the surface of leaded free-machining steel swarf. The swarf surface had contacted the rake face of the tool during machining at a surface speed of 120 feet min⁻¹ (0.61 m s⁻¹). The steel had the following composition: C, 0.10%; Si, 0.01%; Mn, 1.0%; S, 0.30%; Pb, 0.18%; Ni, 0.03%; Cr, 0.01%; Mo, 0.01%; Cu, 0.10%; P, 0.063%; N, 0.004%. The ordinate is a derivative of the electron energy distribution, $n(E)$.

for a total of 1,680 s resulting in further removal of lead, the manganese concentration remaining constant. The profile shows that the surface region of the swarf consists of a series of layers, an outer contamination layer containing carbon, then an extremely thin layer of lead which in turn covers a composite substrate of iron and relatively thick patches of manganese sulphide.

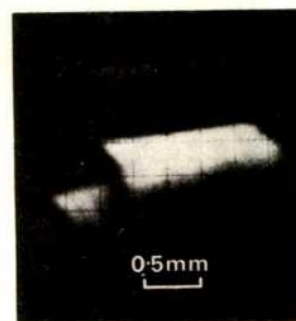


Fig. 2 Scanning electron Auger micrograph (Augergraph) of leaded steel swarf showing uniform distribution of lead (bright areas) on the swarf surface. This structure was obtained by programming the primary electron beam (50 μ m diameter) to describe a raster on the target (as with the scanning electron microscope) and by using the signal from the lead Auger electron peak to modulate the brightness of a video display¹.

The ratio of the observed lead (91 eV) to iron (700 eV) Auger electron peak-to-peak amplitudes ranged from 1.5 to 8.5 for swarfs obtained at cutting speeds between 50 and 350 feet min⁻¹ (0.25 and 1.78 m s⁻¹). The variation in the ratio with cutting speed is the subject of a further investigation. The expected relative peak intensity value for one monolayer of lead on iron is 9.2, calculated from the Auger electron spectra of the pure elements and from measured electron ranges in similar elements^{2,3}. It seems therefore that the lead is concentrated in a layer about one monolayer thick. Further evidence for this localisation is obtained from the shape of the sputtering profile. The predicted profile from sputtering statistics for one monolayer or fraction of a monolayer of lead on iron is of the form $\exp(-nyt)$ where n is the number of argon ions per target atom per second, y is the sputtering yield and t is time¹. Figure 4 shows the observed exponential decrease with ion bombardment time.

To obtain the precise thickness of the lead layer from the profile it is necessary to know the relevant sputtering yield. The appropriate sputtering yield in this case is that for lead from the surface of iron, both elements having been oxidised by air exposure; this yield is not known. It may be expected to differ from the value for bulk lead since Tarng and Wehner⁴ have shown that the sputtering yield of an atom is strongly dependent on the substrate. From the ion beam current density ($n=1.2 \times 10^{-2}$ ions per lead atom per second) a value of $y=0.35$ would be consistent with a monolayer lead level. As expected, this value is lower than the yield for bulk lead since yields are

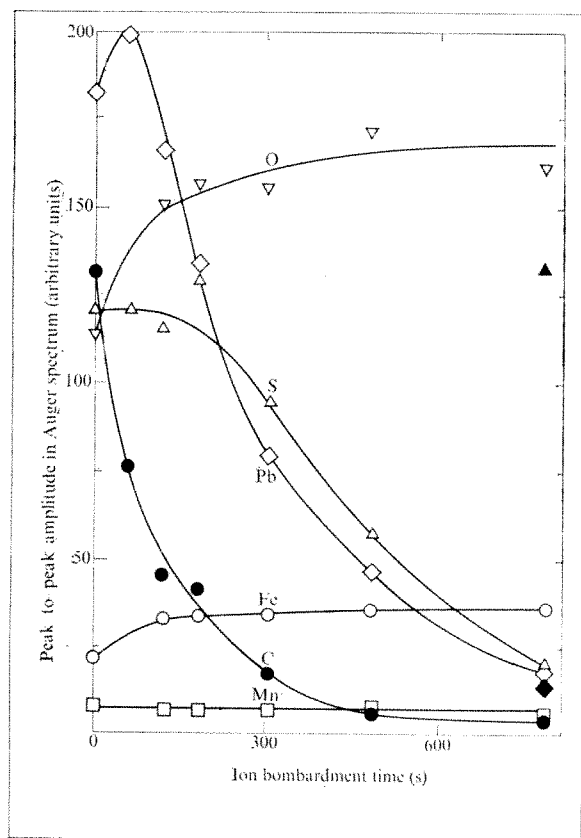


Fig. 3 Element concentrations normal to the surface of the swarf described in Fig. 1. The profile was obtained by sputtering at normal incidence with 620 V argon ions and monitoring with AES. The Auger electron peaks used were those shown in Fig. 1; in the case of iron and manganese the peaks used are marked with an asterisk. The filled symbols (\blacktriangle and \blacklozenge) on the right of the figure refer to sulphur and lead after moving the electron beam to a neighbouring position on the ion etched surface (see text).

inversely proportional to heats of sublimation⁵, and surface energy measurements indicate that lead adsorbs strongly on the surface of iron⁶.

It is considered that the lead layer is formed on the surface of the swarf during cutting by a process of extrusion due to the high compressive forces in the primary deformation zone. The movement of lead would also be assisted by the high local temperatures generated during cutting. It is important to note that not more than one monolayer of lead is formed on the swarf over the range of cutting speeds investigated. This may be due to the higher binding energy of the first layer of lead atoms to the iron substrate, compared with the second layer of lead atoms to the first.

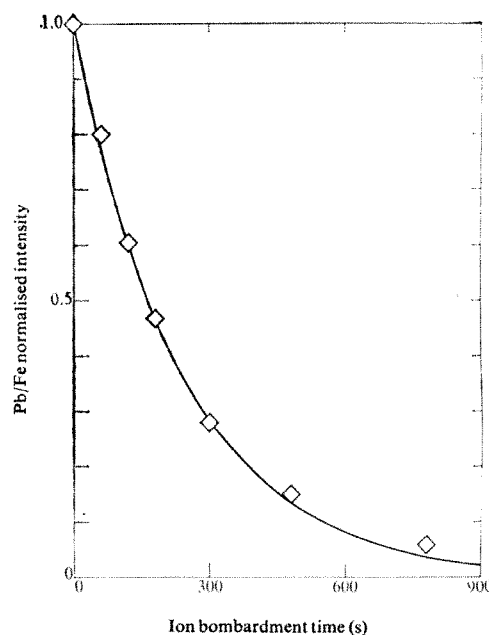


Fig. 4 Sputter profile normal to the swarf surface for lead relative to iron derived from Fig. 3. The curve is the exponential fit described in the text.

The wear mechanisms relevant to cutting tool wear and tool failure have been reviewed by Tabor⁷, the operative wear mechanisms depending critically on temperature and the properties of tool and workpiece. Wear processes of M2 high speed steel during the machining of a wide range of free-machining steels have recently been studied (S. Akhtar and B. M., unpublished), confirming that the presence of lead in resulphurised free-machining steels gives an additional improvement in cutting tool life above that conferred by manganese sulphide alone. Their study has also confirmed that the principal wear mechanisms in the cutting range 50 to 325 feet min⁻¹ (0.25 and 1.65 m s⁻¹), using high speed steel cutting tools, are adhesive and abrasive wear. It would seem that a layer of lead on the swarf at this surprisingly low monolayer level is sufficient lubricant to give improved tool life by the reduction of adhesive wear. It is known that the equilibrium segregation of a minority species to monolayer levels at grain boundaries leads to reduced grain boundary cohesion⁸ and that lead can induce brittle failure of high strength steels⁹. Similarities may exist in the present reduced adhesion between the swarf from leaded free-machining steel and the tool.

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Lattice resolution in an electron-beam sensitive polymer

RECENT developments in our understanding and application of high-resolution defocusing phase contrast electron microscopy have contributed significantly to structural studies of carbon fibres from a range of precursor materials¹⁻⁴. For example, we are now able to follow the natural pattern of carbonisation in the standard commercial process for polyacrylonitrile (PAN)-based carbon fibres, and to detect variations in structure which give rise to abnormal physical properties^{5,6}. One major factor which has influenced the success of the work on carbon fibres is the stability of the stacked carbon ribbons to irradiation by an electron beam, and the relative ease with which the {002} lattice fringes can be recorded. In fibrous organic polymers we have observed massive degradation of structural order as revealed by the rapid deterioration of the electron-diffraction pattern^{7,8}; this has precluded the recording of lattice-fringe images and so prevented the breakthrough in understanding which would undoubtedly follow as a result of direct observation of fibre structure at the molecular level.

Here we report for the first time the direct imaging of crystallite lattice fringes in a beam-sensitive polymer together with a preliminary analysis of diffraction data in terms of crystallite size.

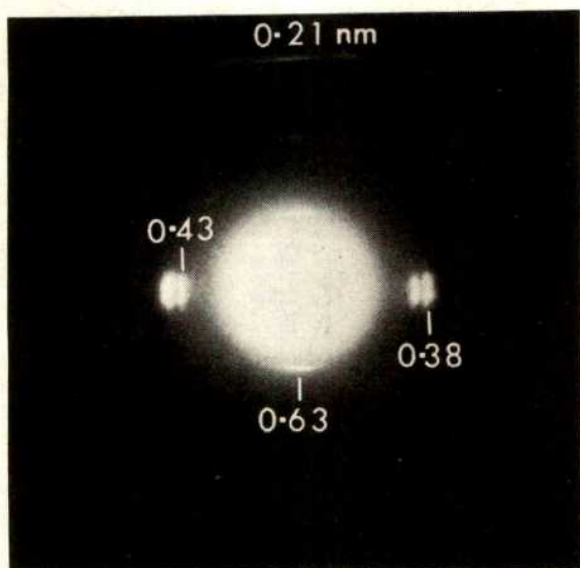


Fig. 1 Electron diffraction pattern from Kevlar 49.

Table 1 Crystallite size in Kevlar 49

Technique	Crystallite size (nm)
X-ray diffraction	7.9
Electron diffraction	8.1
Lattice fringes	8.6-22.5

Recently, E. I. du Pont de Nemours and Co. Inc. have developed the poly-(*p*-phenylene terephthalamide) fibre Kevlar 49 composed of hydrogen-bonded sheets containing phenylene rings, whose mechanical properties approach those of PAN-based intermediate-modulus carbon fibres⁹. A preliminary X-ray diffraction survey indicates that the material is highly crystalline and there is no evidence for the small-angle meridional reflections which are traditionally associated with chain folding in polyamide and polyester

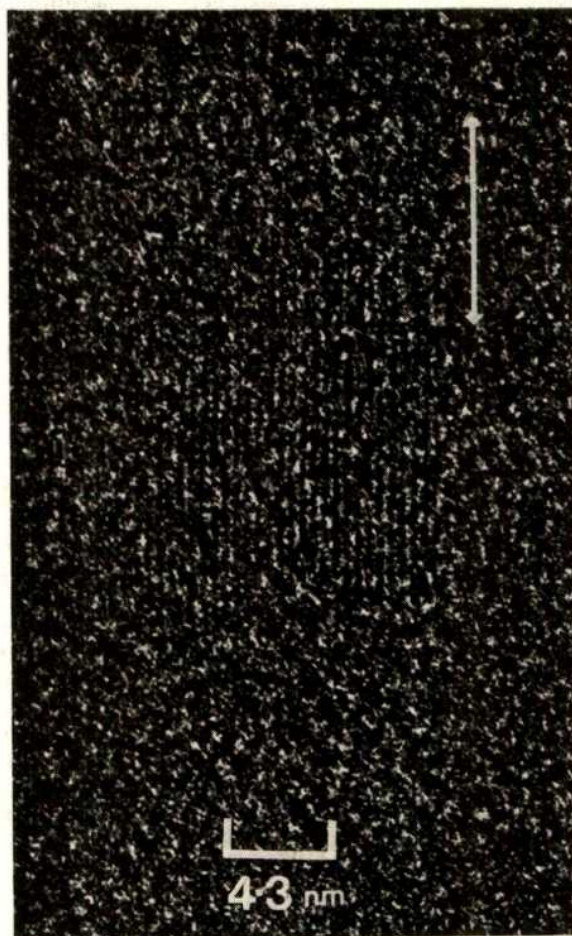


Fig. 2 Micrograph showing 0.433 nm lattice fringes in Kevlar 49 ($\times 3,200,000$).

fibres. Quantitative analysis of the equatorial X-ray diffraction trace by a method of profile resolution developed earlier¹⁰, indicates a crystallite width of 7.9 nm normal to the fibre axis, as obtained from the width of the resolved 0.433 nm reflection. The usual corrections were made to the overall intensity profile obtained by a step-scan diffraction procedure, and, after resolution, the peaks were corrected for instrumental broadening by a Stokes deconvolution method.

For electron-microscope examination in a Philips EM300, fibres of Kevlar 49 were fragmented by ultrasonic irradiation and the resulting material deposited on carbon-coated grids in the usual way. Electron diffraction patterns were

recorded which show a wealth of reflections. The principal diffraction maxima are shown in Fig. 1 where the innermost equatorial and three meridional reflections (even-order layer lines) correspond to Bragg spacings of 0.433, 0.388 nm, and 0.634, 0.317, 0.211 nm, respectively. These reflections decay at different rates during exposure to the electron beam; the 0.433 nm reflection is most stable but does not persist longer than about 120 s at a beam density of 3.28×10^{-4} A mm⁻². The equatorial intensity distribution was obtained from a corrected microdensitometer trace and analysed by a modified profile-resolution procedure developed for electron-diffraction analysis of selected areas in carbon fibres of heterogeneous structure¹¹. In spite of some degradation in the electron beam, the average crystallite size obtained from the breadth of the resolved 0.433 nm reflection is 8.1 nm, a value which compares favourably with the value obtained from X-ray diffraction analysis (Table 1).

Medium-resolution electron micrographs reveal breakdown of the fibres into extensive sheets which exhibit numerous kink bands, and, under dark-field conditions, a dense population of crystallites. Using carefully selected instrumental operating conditions developed from work on other beam-sensitive polymers⁷, high-resolution electron micrographs were recorded at magnifications calibrated by measurement of the 1.194 nm lattice fringes derived from the (201) planes in platinum phthalocyanine crystals. At the appropriate focal level, micrographs of Kevlar 49 clearly show lattice fringes related to the layer planes from which the 0.433 nm equatorial reflection originates; an example is illustrated in Fig 2. Although it must be emphasised that there is no exact one-to-one correspondence between layer planes and lattice fringes, an estimate of crystallite size in a direction at right angles to the fibre axis can be determined directly from such micrographs. Figure 2 shows an array of almost parallel lattice fringes approximately 12.5 nm in width, whereas in other micrographs more disordered lattices are apparent.

Measurement from a number of micrographs gives a size distribution within the range 8.6–22.5 nm (Table 1). It would appear that the averaged values obtained from fragments and fibres of Kevlar 49 are below the range measured directly. This comparison immediately questions the quantitative estimation of crystallite size by the classical diffraction methods, although it is possible that at present our lattice fringes are derived from the more perfect regions of the structure.

In conclusion we believe that the importance of this work is twofold. First, it provides a basis for the elucidation of relationships between molecular structure and physical properties in polymer systems, and second, it should enable us to test the validity of standard diffraction methods used to assess crystallite size and order.

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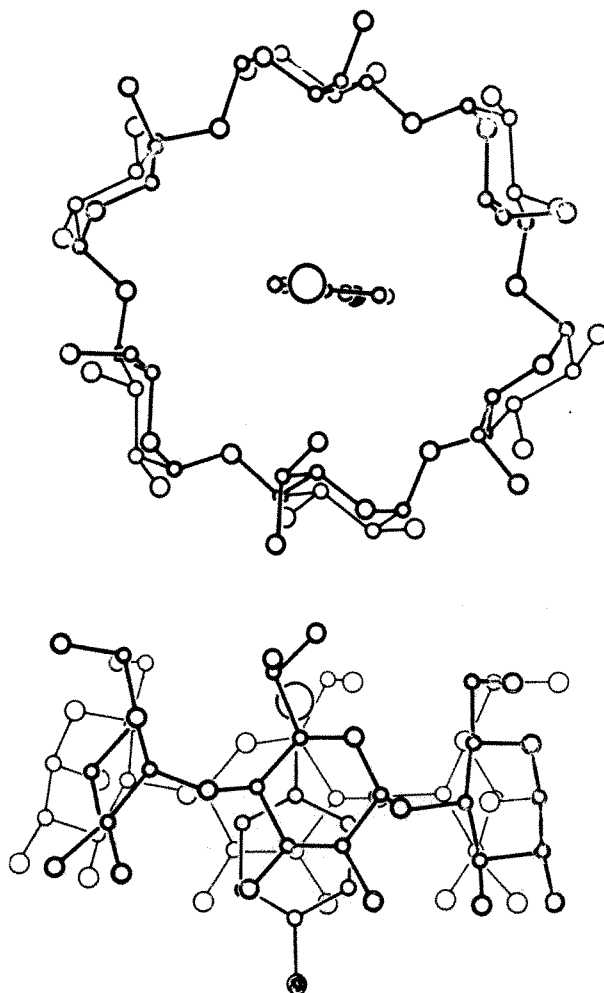
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Structure of α -cyclodextrin-*p*-iodoaniline complex

CYCLODEXTRINS, which are cyclic α -1,4-linked D-glucose oligomers, form a number of inclusion compounds with a variety of 'guest' molecules¹. The direct evidences for the formation of inclusion compounds have been derived from NMR spectra² and X-ray studies^{3–5}. α -Cyclodextrin is capable of forming a complex with a bulky molecule whose length is longer than the depth of the cavity of α -cyclodextrin. It is as yet not clear that which part of the molecule is included in and in what manner the molecule is bound to the interior of the α -cyclodextrin ring. We present here the structure of the α -cyclodextrin-*p*-iodoaniline (α -CDx-*p*-IAN) complex, (C₆H₁₀O₅)₆·C₆H₄NI₃H₂O.

The crystal is orthorhombic and the space group is P2₁2₁2₁, with unit cell dimensions; *a*, 13.659 (2) Å; *b*, 15.413 (4) Å; *c*, 24.436 (5) Å. The calculated density with *Z* = 4 was 1.610 g cm⁻³ and the observed density was 1.602 g cm⁻³ (flotation method). Intensities for 5,007 independent reflec-

Fig. 1 The structure of the α -cyclodextrin-*p*-iodoaniline complex. The circles, in order of decreasing size, represent iodine, oxygen and carbon atoms, and the black dots indicate nitrogen atoms.



tions were measured on a Rigaku AFC four-circle diffractometer using graphite monochromatised MoK α radiation ($2\theta \leq 50^\circ$).

The structure was solved by the heavy atom method using 1,171 reflections with $2\theta \leq 30^\circ$. The three-dimensional electron density map calculated on the basis of coordinates of the iodine atom revealed the rough structure of the complex. The least-squares refinement of the atomic parameters which were estimated on the electron density map was unsuccessful. The accurate positions and directions of the six glucose units and the *p*-IAN molecule were determined by the block-diagonal rigid-body least-squares technique³. The O₆ atoms and water molecules were found on a difference Fourier synthesis. In successive refinements, one of the O₆ atoms was found to be disordered. Using 3,519 reflections with $|F_o| \leq 3\sigma(F)$, the final block-diagonal least-squares refinement with anisotropic temperature factors reduced the *R* value to 0.072. Fig 1 illustrates the structure of the complex.

Each glucose unit is in the C1 chair conformation, and all glucose units are α -1,4-linked; this result is in agreement with the structures of the other α -CDx complexes which were determined by X-ray analysis³⁻⁵. The *p*-IAN molecule is included in the cavity along the axis of α -CDx. The iodine atom and the benzene ring are situated in the cavity of α -CDx, while the nitrogen atom lies outside the cavity. The shortest distance between the benzene ring and α -CDx is 3.25 Å which is from an O₄ atom to a carbon atom of the benzene ring. This rather short intermolecular distance indicates that *p*-IAN is rigidly fixed in the cavity. The shortest distance from the iodine atom is 3.70 Å which is the distance to the disordered O₆ atom.

In the α -CDx-*p*-IAN complex, the six O₄ atoms of α -CDx lie very near the least-squares plane of themselves, and the maximum deviation from the plane is 0.13 Å. In the α -CDx-potassium acetate complex³ and the α -CDx-H₂O complex⁴, the maximum deviations are 0.01 Å and 0.98 Å, respectively. The average valence angle of α -1,4-linking oxygen atoms is 120° (2) which is in agreement with 119.1° in the α -CDx-potassium acetate complex and $119 \pm 3^\circ$ in the α -CDx-I₂ complex⁵. The distances between oxygen atoms O₂ and O₃ of adjacent glucose units are 2.68, 2.76, 3.00, 2.92, 2.98 and 2.87 Å which are the acceptable distances for hydrogen bonds. On the other hand, in the α -CDx-H₂O complex and the α -CDx-I₂ complex, there exist distances of 3.36 Å and 3.83 Å, respectively. In the α -CDx-*p*-IAN complex, all C₆-O₆ bonds are oriented to the outside of the cavity and the conformation is the same as that found in the α -CDx-I₂ complex; the conformational angles O₅-C₅-C₆-O₆ are $-75 \pm 8^\circ$ in the α -CDx-*p*-IAN complex and $-70 \pm 8^\circ$ in the α -CDx-I₂ complex.

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Is the aardwolf a mimic of the hyaena?

MIMICRY is a phenomenon of evolutionary convergence or parallelism by which an edible mimic species gains some measure of protection from predators by virtue of its close

resemblance to a model species which is unpalatable (that is, distasteful or dangerous)¹⁻³. Predators learn to avoid the unpalatable model species after one or more adverse experiences with it, and any mimic species that resembles the model sufficiently closely will likewise be avoided.

The first examples of mimicry were documented in Brazilian butterflies¹—most additional examples and experimental work have been limited to mimicry in insects. Vertebrates exhibiting mimicry are rare, but include fish⁴, snakes^{5,6} and birds^{7,8}. A single example has previously been described in mammals: Shelford described a series of five squirrel species which mimic unpalatable treeshrew models in Borneo^{9,10}. The case described here is a possible additional example which, if confirmed, extends the phenomenon of mimicry to a large African mammal.

The genus *Hyaena* is represented in Africa today by two species: the striped hyaena, *H. hyaena*, inhabiting northern Africa; and the brown hyaena, *H. brunnea*, limited to southern Africa. Hyaenas (the spotted hyaena, *Crocuta*, excluded) weigh 50–60 kg, and inhabit open dry plains and thorn scrub, live singly or in pairs, and are chiefly nocturnal. *Hyaena* has a sloping back, pointed ears, and an erectile mane. It has strong

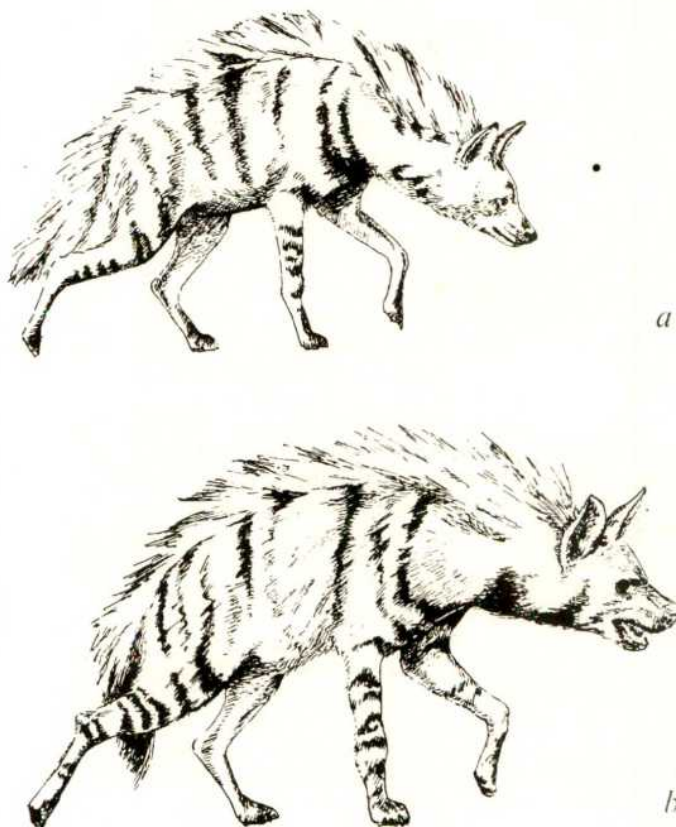


Fig. 1 External appearance of, a, the aardwolf, *Proteles cristatus*, and, b, the hyaena, *Hyaena hyaena*, both drawn to the same scale. Redrawn by P. Olsen from colour illustrations in Dorst and Dandelot²⁰.

teeth and jaws and it is known to kill live game occasionally, although it is primarily a scavenger.

The aardwolf *Proteles cristatus* is a much smaller, jackel-sized (12–15 kg) mammal with a discontinuous distribution: a northern population is found throughout most of East Africa, and a separate population inhabits southern Africa. *Proteles*, like *Hyaena*, has a sloping back, pointed ears, and a well developed erectile mane along the dorsal spine from neck to tail. This mane is composed of stiff hairs some 20 cm in length and, when erected, makes the aardwolf appear considerably larger than it actually is¹¹. *Proteles* inhabits open

plains and thorn scrub, lives singly or in pairs, and is chiefly nocturnal. It has only vestigial cheek teeth and feeds largely on termites and other insects, and occasionally on carrion and rodents^{12,13}.

In his initial description of *Proteles*, Geoffroy St-Hilaire¹⁴ noted that its external appearance is very similar to that of *Hyaena*, a resemblance noted in virtually every subsequent description of the aardwolf (Fig. 1). Kruuk¹³ observed that *Proteles* and *Hyaena* look so similar that they are often confused. The resemblance of *Proteles* to *Hyaena* in body, mane and tail colour, and in the colour and development of stripes remains close in spite of considerable clinal variation in these characters; the pattern in each subspecies of *Proteles* resembling closely the sympatric subspecies of *Hyaena*¹⁴⁻¹⁶. Size is the only real difference in the external morphology of the two, and this is a notoriously difficult quantity to perceive in the field in the absence of some fixed comparative scale.

In addition, *Proteles* is found throughout its distribution in the same open plain or thorn scrub habitat as *Hyaena*, and its general behaviour is remarkably similar. The aardwolf and hyaena are both chiefly nocturnal, and live singly or in pairs. Even their kneeling defensive response to attack is similar¹¹.

The external similarity of *Proteles* to *Hyaena* extends also to most of the characters of internal anatomy that have been studied, indicating that *Proteles* is closely related to the hyaenas. The chromosomes¹⁷⁻¹⁹ and haemoglobin mobility²⁰ of *Proteles* are virtually identical to those of *Hyaena* and *Crocota*, and the gyri and sulci of the cerebral hemispheres of the brain are arranged on exactly the same plan¹¹. The complete dental formula of *Proteles*, though not always fully developed, is the same as that of *Hyaena* and *Crocota*²¹. The male reproductive tract of *Proteles* appears to be more similar to that of *Hyaena* than to that of *Crocota*^{11,22}. *Proteles* differs from *Hyaena* principally in having a dentition much reduced in size, and in retaining the pollex (a digit lost in both *Hyaena* and *Crocota*)²³.

The evolutionary lines leading to *Hyaena* and *Crocota* appear, from the fossil record, to have been distinct since the Miocene²⁴. Fossils bearing on the evolution of the aardwolf have recently been discovered^{21,25} which indicate a Pliocene or earlier time of divergence of *Proteles* from the ancestral hyaenid stock, but the precise phylogenetic relationships among these three genera of Hyaenidae are not yet completely clear.

Two hypotheses of hyaenid relationships seem tenable. *Proteles* may have separated from the common ancestor of *Crocota* and *Hyaena* before they separated (suggested by the shared loss of the pollex in the latter two genera), in which case the close external resemblance of *Proteles* to *Hyaena* is perhaps convergent. On the other hand, *Crocota* may have separated from the *Proteles*-*Hyaena* stock before these two became differentiated (suggested by the reproductive tract and the close external resemblance), in which case the external resemblance of *Proteles* and *Hyaena* may reflect a parallel retention from a common ancestor having this appearance.

The nearly complete geographic sympatry, close external resemblance, and similar behaviour of *Proteles* and *Hyaena* are unusual in closely related forms, since speciation normally involves significant divergence in geographic distribution, in external appearance, or in behaviour. All of the conditions of Batesian mimicry are met by *Proteles*, and this may help to explain its distribution, appearance, and behaviour. Only the predator remains to be identified.

Leopards occur throughout the range of *Proteles*, they are most active at night, and they are predominantly visually oriented predators. Leopards routinely prey on jackals^{26,27} and, in view of their wide prey tolerance²⁸, might be expected to prey on *Proteles* as well. Because of its large size and strong skull, a *Hyaena* would be dangerous to an attacking leopard. The much smaller, weaker *Proteles* would be a poor match for a leopard, and it seems that an important component of the aardwolf's defence against predators may be its close external resemblance to the larger, more dangerous *Hyaena*. The ability of *Proteles* to erect its mane when excited would

further confuse a potential predator as to its actual size and identity.

This possible case of mimicry in a large mammal is unique, and deserves further investigation. Unfortunately, low population densities and nocturnal activity patterns make both *Proteles* and *Hyaena* difficult to study in the field. Additional study of their behaviour and ecology will be of great interest, and observation of any interactions with leopards of particular importance.

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Total human body protein synthesis in relation to protein requirements at various ages

THE intensity of body and tissue protein metabolism per kg declines with increased adult body size in mammals¹. This fall parallels a similar progressive decline in the intensity of energy metabolism²⁻⁴. It has also been concluded that protein metabolism per unit of body weight is about four to five times faster in young rats than in adult man¹; this pattern of change extends to cellular and subcellular aspects of protein metabolism, such as plasma albumin synthesis, liver RNA content and enzyme activity^{1,5}. Similarly, the rate of protein synthesis per kg total body weight declines during growth and development within a species, such as the rat⁶. This parameter again parallels the reduction in the intensity of energy metabolism which occurs during the growth period³.

Such surveys provide a general picture of protein metabolism in mammals and help to explain the metabolic basis for differences in dietary protein requirements both within and between the various species. The concepts involved should apply to man at various ages⁷⁻⁹. In previous studies with human subjects, however, different approaches have been used, some of which are no longer considered valid, and insufficient detail has been provided. It is therefore difficult to use the estimates to compare changes in body protein and energy metabolism with those for dietary protein needs throughout a man's life.

Using a common approach, we have tried to characterise the relationship which exists between protein and energy metabolism in man and to examine dynamic aspects of body protein metabolism with reference to dietary protein needs, during the period of rapid growth and development and the later years when senescence dominates body metabolism and function. The procedures, analytical methods and calculations of total body

protein synthesis have been described previously (refs 11, 12, and W.P.S., P.B.P., R. S. Goldsmith, N.S.S., and V.R.Y., unpublished). To quantitate the rate of total body protein synthesis we used a modification of the constant isotope method¹⁰, which is based on the continuous administration of ¹⁵N-labelled glycine and the analysis of urinary urea for ¹⁵N after a constant level of isotope enrichment has been achieved. The main difference in our experiments was that equal tracer levels of ¹⁵N-glycine were given orally at intervals of 3 h, rather than by continuous intravenous or intragastric infusion. We found that these two methods of infusion (continuous and at

Table 1 Total body protein synthesis rate in humans at various ages

Age group	No. of studies	Body weight (kg)	Age (range)	Total body protein synthesis (g kg ⁻¹ d ⁻¹)
Newborn (premature)	10	1.94±0.59	1-46 d	17.4±7.9
Infant*	4	9.0±0.5	10-20 months	6.9±1.1
Young adult	4	71±15	20-23 yr	3.0±0.2
Elderly	4	56±10	69-91 yr	1.9±0.2

*For comparative purposes, the data of Picou and Taylor-Roberts¹⁰ have been included.

intervals) give essentially identical estimates of total body protein synthesis. ¹⁵N-glycine (95 or 99 atoms per cent excess) was administered at a rate of 0.5 mg ¹⁵N per kg body weight per day, given during a 60-h tracer period in studies with young adults and the elderly.

Three age groups were studied. (1) Newborn, including six premature infants and one full-term baby. All were studied during a 30-36 h ¹⁵N tracer period within 1-45 days of life. One baby was studied at 1 and 13 days of age and another at 16, 31 and 45 days of age. (2) Healthy, normal young adults, three male and one female, 20-23 yr old. (3) Healthy, elderly women, 69-91 yr of age. Diets provided constant intakes of essential nutrients judged to be adequate for each age group. A more detailed description of the subjects, experimental details and diets will be published elsewhere. Urinary urea was isolated¹³ and analysed for ¹⁵N, following reaction with hypobromite¹⁴, with the aid of a dual collector, isotope ratio mass spectrometer (MS 11, Vacuumetrics, Waltham, Massachusetts).

Table 1 summarises results for body protein synthesis for the three age groups studied. The intensity of body protein synthesis declined rapidly during the first year of life, the rate in young adults being about one sixth that in premature infants. During later adult years protein synthesis (per unit of body weight) continued to decline but more gradually, the value for elderly subjects being 63% that for young adults. The differences among the older age groups reflect, in part, body compositional differences but this could not account for the marked decline in the synthesis rate between premature babies, full-term infants and young adults. Thus, as observed in experimental animals⁶ the intensity of protein metabolism in man declines throughout life.

To explore further the relationship between changes in the intensity of whole body protein synthesis and in energy metabolism we have recalculated the data on the basis of energy expenditure for each age group. For the prematures total energy intake was used for the calculation; for comparing adults, it was considered appropriate to use the basal metabolic rate. The results are summarised in Table 2. Differences observed in the intensity of protein metabolism among the various age groups (Table 1) are essentially eliminated when body protein synthesis is related to the energy expenditure for the different age groups (Table 2). These results confirm previous observations that protein metabolism is closely related to basal energy metabolism both within and between mammalian species^{1,4}.

Finally, the nutritional significance of these observations on whole body protein synthesis may also be assessed by comparing changes in dietary protein needs at various ages with decline in the intensity of whole body protein metabolism. The comparison

Table 2 Mean total body protein synthesis in relation to energy metabolism and Dietary protein allowances in premature babies, infants, young adults and elderly women

Age group	Dietary protein allowance* (g kg ⁻¹ d ⁻¹)	Protein synthesis (g (per caloriet) (per g protein allowance)
Newborn (premature)	3.2	0.15±0.09†
Infants§ (~1 yr)	1.3	5.3
Young Adult	0.57	0.11±0.01
Elderly	0.42	0.11±0.03

*Values for prematures are based on mean protein intake by the babies studied. Allowances for infants and young adults taken from FAO/WHO¹⁵ and for the elderly the allowance is based on the same method of estimation using unpublished data (W. D. Perera), on determined obligatory urinary and faecal nitrogen losses for these four subjects.

†Energy expenditure for prematures based on total energy intake. For young adults the values for basal metabolic rate were obtained by calculation from Altman and Dittmer¹⁶. In the elderly group basal metabolic rate was determined by indirect calorimetry.

‡Mean ± s. d.

§Values for protein synthesis taken from Picou and Taylor-Roberts¹⁰ for this age group.

provided in Table 2, using current estimates for daily protein needs¹⁵, shows a fall in the latter which parallels that for total body protein synthesis with progressive growth and during subsequent ageing. Hence, the amount of utilisable protein required to support body protein synthesis does not show a marked age-dependent change in man when needs are related to protein synthesis. Our data indicate that approximately 1 g of dietary protein is required to support the needs for 4 to 5 g of total body protein synthesis. This suggests that the efficiency of total dietary nitrogen utilisation is similar in the newborn, adult and elderly, and that the differences in protein needs, expressed per kg body weight, for the various age groups are related to differences in the amount of protein synthesised per unit time.

This conclusion may also apply equally to the individual dietary essential amino acids, except during the early neonatal period when histidine¹⁷ and cystine¹⁸ appear to be required as obligatory constituents of the diet because the enzymes responsible for endogenous synthesis, at rates commensurate with metabolic needs, have not fully matured.

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Mechanisms of cell fusion

We report new findings on the chemically-induced fusion of hen erythrocytes and discuss possible ways in which the lipid and protein/glycoprotein components of membranes may behave during the process of membrane fusion. Our observations are consistent with Poste and Allison's¹ idea that the aggregation of intrinsic membrane proteins is important in membrane fusion but we suggest that fusion may occur in regions of perturbed lipid bilayer which are thus denuded of intramembranous particles.

The intramembranous particles of unfixed T and B mouse lymphocytes have recently been reported to aggregate on incubation at 0°C in dimethyl sulphoxide (DMSO) or 25-50% glycerol²; glycerol-induced aggregation of membrane-intercalated particles has also been observed in *Entamoeba histolytica*³. To test Poste and Allison's hypothesis, we have therefore studied the effects of these agents on hen erythrocytes to determine whether they induce these cells to fuse. On incubation for 5 min at 37°C with 5 M DMSO (35%), the cells became spherical and binucleate cells were observed after 25-30 min. The number of fused cells and the number of nuclei per cell both increased on further incubation (Fig. 1). No fusion occurred on incubation for 45 min with 3 M (or lower concentrations of) DMSO. We anticipated that DMSO would cause cell fusion because it causes the formation of large membrane blisters devoid of intramembranous particles in lymphocytes². The plasma membranes of fibroblasts treated *in vitro* with oleylamine also blister away from the underlying cytoplasmic organelles, and their plasma membranes usually fuse in these regions⁴.

Stewart and Turner⁵ have previously reported, although without comment, an apparent fusion between pairs of human erythrocytes observed in electron micrographs of cells treated with 40% glycerol. In our experiments multinucleated cells were observed on incubation of hen erythrocytes for 22 and 5 h at 37°C in modified Eagle's basal salt medium at pH 7.4 (ref. 6), containing Dextran 60 C (80 mg ml⁻¹) and glycerol (1.5 or 2.5 M, respectively). Dextran 60 C facilitated fusion induced by these quantities of glycerol but in control incubations without glycerol the dextran was not in itself fusogenic⁶. In the absence of dextran most of the glycerol-treated erythrocytes became swollen and were then lysed: only a few cells fused. Fusion induced by DMSO and glycerol, like that induced by fusogenic lipids⁶ was calcium-dependent (1.8 mM Ca²⁺) and was inhibited by EDTA (5 mM).

Since glycerol causes cell fusion, other polyols have been

investigated for fusogenic properties. Sorbitol fuses hen erythrocytes, numerous multinucleated cells being present after 2 h incubation in 2.5 or 3 M sorbitol dissolved in the standard buffer (free from Dextran 60 C). Higher and lower concentrations of sorbitol were less effective. Multinucleated cells have also been observed with mannitol (1.25 M, 6 h incubation), ethylene glycol (20%, 5 h incubation) and sucrose (1 M, 5 h incubation). The non-fusogenic Dextran 60 C (molecular weight approximately 90,000) greatly facilitated fusion by sucrose. In contrast, long incubation (30-48 h) of the cells

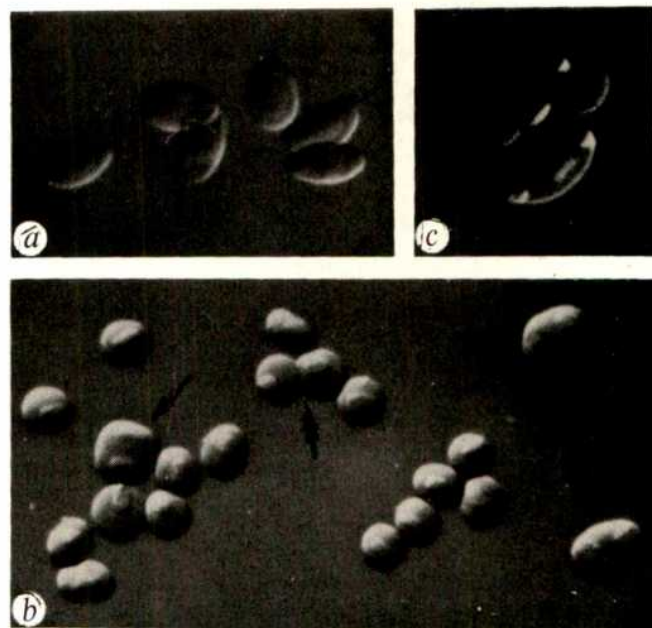


Fig. 1 Light micrographs ($\times 672$) of hen erythrocytes: a, cells (approximately 3.5×10^8 cells ml⁻¹) incubated at 37°C at pH 7.4 in Eagle's basal salt solution buffered with sodium cacodylate⁶; b, cells incubated in the buffer for 30 min at 37°C with 5 M dimethyl sulphoxide (DMSO), showing the cell adhesion which precedes cell fusion (double arrow) and three binucleate cells (single arrows); c, a multinucleated cell after incubation for 90 min as in b. Nomarski differential-interference-contrast microscopy.

with 80-100 mg ml⁻¹ of a high molecular weight dextran (2×10^6) was itself capable of yielding many multinucleated cells. Polyethylene glycol (molecular weight 6,000), which has recently been shown to fuse plant protoplasts⁷, is also capable of causing extensive cell fusion with hen erythrocytes.

Aggregation of membrane proteins may occur either by disorganising membrane lipids with a micellising agent such as lysolecithin⁸, or by increasing the fluidity of membrane lipids by heating⁹, or by the insertion of a low melting fatty acid or ester⁶. Whether glycerol induces protein clustering by interacting with the lipids or the proteins of membranes is problematic. The addition of glycerol to a lipid film at an air-water interface will, however, lower the surface potential of a condensed or an expanded film and also further expand a partially-expanded film¹⁰.

Poste and Allison¹ indicate that complete fusion of membranes, as observed in cell fusion, may be regarded as an extension of partial fusion observed in intercellular junctions. Particle aggregation in junctions presumably involves stable intermembranous protein-protein interactions as well as intramembranous protein clustering. In contrast, only the latter may be significant in membrane fusion, and protein clustering may be followed by lipid-lipid interactions between membranes, leading to a loss of membrane individuality and allowing membrane fusion to proceed. This interpretation is consistent with the fact that sonicated protein-free phospholipid vesicles can fuse readily under specified conditions¹¹⁻¹³.

Whether or not endogenous enzymes (for example, lipase¹⁴) are involved in one or more stages of chemically- or virus-

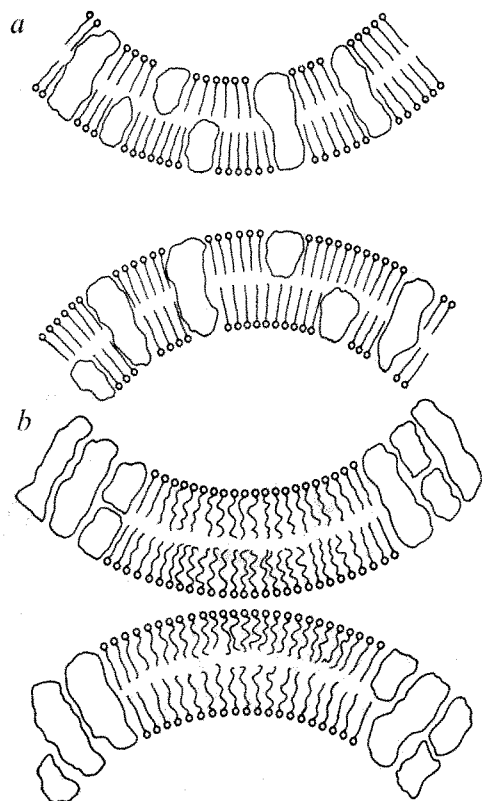


Fig. 2 Possible relationships between the lipid molecules and the intrinsic proteins of biological membranes during membrane fusion: *a*, two membranes in close proximity which do not fuse because the proteins of the membranes are randomly arranged in their respective lipid bilayers; *b*, membranes in which fusion may now proceed, by the interaction and intermingling of their lipid molecules, after the emergence of protein-free areas of lipid bilayer as a result of aggregation of the intrinsic proteins in both membranes. Compared with *a*, the lipid molecules of the membranes that are about to fuse have been perturbed; the lipids of *b*, are either more fluid (as illustrated), or, in the extreme case, are rearranged in micellar form (not shown).

induced cell fusion remains debatable. We suggest therefore that cell fusion induced by exogenous chemical agents seems to involve, *inter alia*, the following events. First, a perturbation of the bilayer structure of membrane lipids may occur which increases the fluidity of the lipid region, or in the extreme case results in micelle formation. These changes in the lipid structure of the plasma membrane then allow the aggregation of intramembranous protein/glycoprotein particles. Second, the interaction and intermixing of the disturbed lipid molecules of closely apposed membranes in regions denuded of intramembranous proteins and glycoproteins may allow adjacent cells to fuse (Fig. 2). Concanavalin A, polylysine and protamine, which have been reported to induce aggregation of membrane proteins, cause erythrocytes to aggregate but not to fuse. In some instances, however, the induction of protein clustering may be sufficient to lead to fusion (such as that of *Drosophila* cells induced by concanavalin A, ref. 15), if aggregation of the proteins gave rise to perturbed, rather than stable, pools of lipid bilayer.

Comparable mechanisms to that suggested here may apply to fusion involving the membranes of subcellular organelles. For example, during mucocyst secretion by exocytosis in *Tetrahymena*, fusion occurs between a region of plasma membrane, sequestered within a rosette of particles, and a smooth area of mucocyst membrane free from particles¹⁶. The complex sequence of changes observed in mucocyst secretion may therefore be a specialised means of allowing the lipid bilayers of closely apposed membranes to interact in regions denuded of intramembranous proteins.

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Infection of differentiating muscle cells with influenza and Newcastle disease viruses

REPLICATION of influenza viruses is believed to require functions provided by the host cell nucleus because first, influenza replication is inhibited by actinomycin D and α amanitin, which prevent transcription of complementary RNA from virion RNA in the first few hours following infection¹⁻⁴; and second, migration of viral proteins between host cell cytoplasm and nucleus occurs during the virus replicative cycle⁵⁻⁸.

We therefore examined the replication of influenza viruses in skeletal muscle cells, as the latter undergo a characteristic differentiation resulting in the formation of syncytial myotubes, the nuclear DNA synthesis of which has terminated^{9,10}, and the RNA metabolism of which has changed considerably¹¹⁻¹⁴. Such myotubes have been reported to be resistant to direct infection by the DNA viruses polyoma and SV40 (ref. 15) and the RNA Rous sarcoma virus¹⁶, all of which interact with host nuclear DNA during their replication.

Breast muscle from 12-d chick embryos was treated with trypsin and cells were cultured in 60 mm Petri dishes as previously described¹⁷. The appearance of uninfected cell cultures during the course of differentiation is illustrated in Fig. 1. One day after plating, cultures contained predominantly dividing single cells, myoblasts (Fig. 1a), and low frequency myotube formation was also observed. This was followed one day later by a period of rapid cell fusion, which had ceased by about 40 h after plating. If carefully selected batches of horse serum were employed, about 70 to 80% of the myoblasts fused (Fig. 1b). After plating for 3 d the myotubes broadened and areas between myotubes filled with a population of single cells which had failed to fuse (Fig. 1c). The myotubes later became striated, and started contracting spontaneously (not shown).

Influenza virus strain A/AA/6/60(H2N2) was studied, using Newcastle disease virus (NDV) as control, as NDV is not believed to require any host nuclear genetic information for its replication (reviewed in ref. 18). Both viruses were passaged in chicken eggs, and virus seeds received were similarly propagated initially in the allantoic cavity of eggs. Subsequently, the viruses were grown in monolayers of primary chick kidney

cells¹⁹ to obtain tissue culture-grown virus stocks which were used to infect muscle cell cultures.

For infection of muscle cell cultures the growth medium was removed, cells were washed twice with Hanks' balanced salt solution (HBSS) and 1 ml of inoculum added. After 30 min adsorption at room temperature and removal of residual inoculum by washing twice with HBSS, growth medium containing 5% horse serum added. Cultures were incubated at 40°C, and examined for cytopathic effect and also for their ability to adsorb chicken red blood cells (haemadsorption).

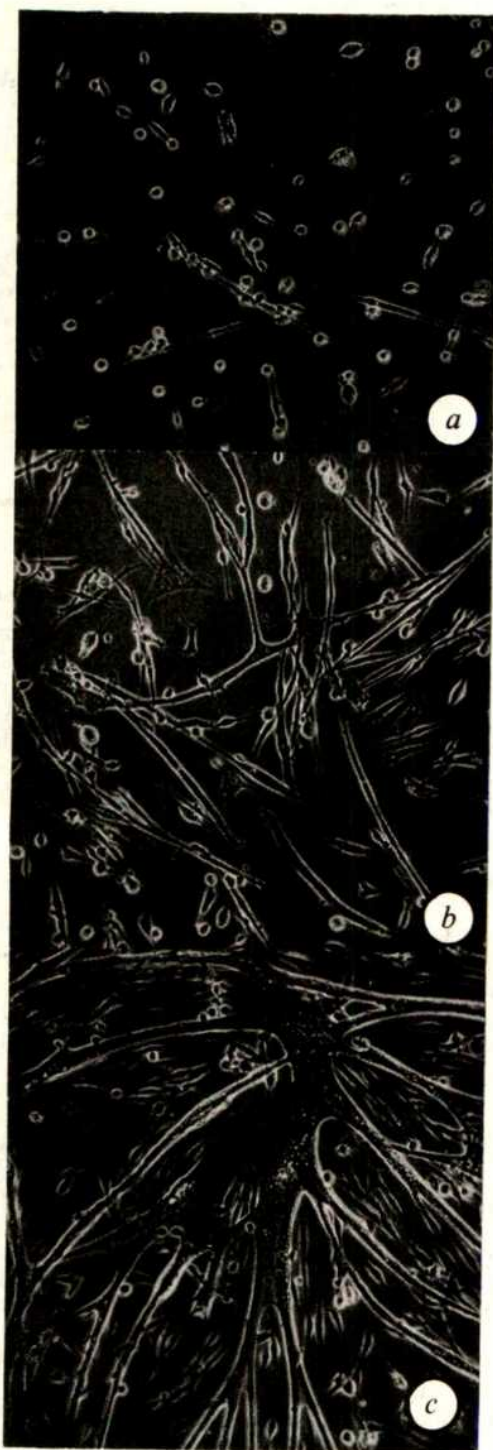


Fig. 1 Kinetics of myotube formation in cultured chicken skeletal muscle cells. *a*, Single cells present in prefusion culture 25 h after plating. *b*, Syncytial myotubes and residual single cells in post-fusion cultures 40 h after plating. *c*, Enlarged myotubes and residual single cells 60 h after plating. Small refractile cells in each case are residual chicken red blood cells after these uninfected cultures were tested for haemadsorption.

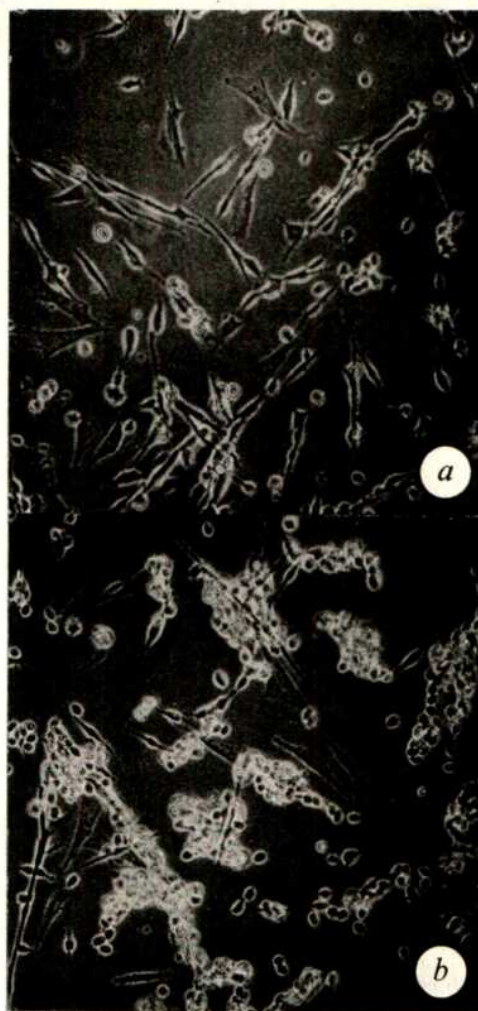


Fig. 2 Lack of influenza replication in prefusion muscle cell cultures. Cultures of skeletal muscle cells were infected 17 h after plating with influenza virus A/AA/6/60(H2N2) or NDV (La Sota) at a multiplicity of 5 to 10 EID₅₀ per cell. After infection for 9 h, cultures were flooded with a suspension of chicken red blood cells, and 10 min later unadsorbed red blood cells removed by gentle washing. *a*, HAD⁻ appearance of culture infected with influenza virus. *b*, Culture infected with NDV having many HAD⁺ single cells and early myotubes. Several identified fields were examined before and after haemadsorption, thereby confirming that many of the HAD⁺ single cells were of myoblast type. Similar results to those shown in the figure were obtained using multiplicities of infection ten times greater.

Figure 2 shows cultures infected with influenza and NDV when observed for haemadsorption 9 h after infection. With the former, very few, if any, single cells were haemadsorption-positive (HAD⁺) in contrast to the NDV cultures where a large proportion of single cells and early myotubes were very strongly HAD⁺. Very similar qualitative findings were obtained in cultures 24 h after infection (not shown).

Table 1 shows this difference in ability of the two viruses studied to replicate in prefusion muscle cells. At 9 h after infection with influenza virus 2% of the cells could be scored as HAD⁺, compared with 1.4% for the control cultures; 24 h after infection with influenza virus 0.8% of the cells could be scored as HAD⁺, compared with 0.26% for the control cultures. It is difficult to interpret this slight difference between values for control and infected prefusion cell cultures as a result of virus replication, in view of the uncertainty with which clusters as small as three red blood cells may be unequivocally identified, and the possibility that small amounts of residual virus from the initial inoculum might cause low levels of haemadsorption. From the results in Table 1 we conclude that any replication of the influenza virus in myoblasts was at best marginal.

Table 1 Efficiency of infection of prefusion muscle cell culture by influenza and Newcastle disease viruses (NDV)

Virus	Time after infection (h)	Total cells	HAD ⁺ cells	% Cells HAD ⁺	% Cells HAD ⁺ , difference from control
None (= control)	9	440	6	1.4	—
None (= control)	24	390	1	0.26	—
Influenza virus A/AA/6/60	9	450	9	2	0.6
Influenza virus A/AA/6/60	24	640	5	0.78	0.52
NDV (La Sota)	9	380	190	50	48.6
NDV (La Sota)	24	420	110	26	25.7

Cells infected with 5 to 10 EID₅₀ of virus were incubated for the time shown before examination for haemadsorption. Each result shown is the total from three or four fields, examined in two separate experiments. Cells were scored as HAD⁺ if they were associated with clusters of three or more red blood cells. Control cells were included to provide a baseline, as small clusters of red blood cells were observed at low frequency in control cultures.

In contrast, NDV exhibited a very significant degree of replication in myoblasts; 9 h after infection 50% of the total cells were HAD⁺ ($P < 0.001$, compared with control or influenza-infected cultures). It should be emphasised that for calculations in Table 1 each myotube was scored as a single cell, although the syncytial cell had been formed by the fusion of some 10 to 20 myoblasts. Accordingly the efficiency of infection calculated for NDV is probably an underestimate, being biased by the fact that the HAD⁺ early myotubes in the cultures were scored

as single cells. Reduction in the proportion of HAD⁺ cells in cultures infected with NDV by 24 h after infection (Table 1) may therefore be an artefact resulting from the increasing degree of cell fusion that occurs in the cultures with time. Even without taking this factor into account, the results in Table 1 show that the efficiency of replication by NDV in myoblast cultures is at least 50 to 80 times greater than for the influenza virus. This has been confirmed by dose-response experiments (M.C.O'N., and A.P.K., unpublished).

We also examined replication by the myxoviruses in post-fusion muscle cell cultures, and for this purpose cultures were maintained without infection until myotube formation had reached its maximum. Infection with influenza virus and NDV was then performed in an identical manner to that employed with prefusion cell cultures. Figure 3a illustrates the high frequency with which myotubes supported influenza virus replication. When cultures were examined about 9 h after infection; virtually every myotube was HAD⁺, whereas of the single cells still present in the culture less than 5% were HAD⁺. Many myotubes and single cells were also HAD⁺ in post-fusion cultures infected with NDV (Fig. 3b).

Similar post-fusion cell cultures were maintained for 24 h after infection with influenza virus or NDV. In this case, influenza virus infected cultures were almost totally devoid of myotubes, which detached from the cell layer, and were observed floating in the culture medium. Since it had been shown that myotubes were infected with influenza (Fig. 3a) it was concluded that prolonged incubation of myotubes after influenza infection resulted in a cytopathic effect and probably cell death. This was supported by the observation that, in post-fusion cultures examined 24 h after influenza virus infection, a few remaining myotubes were strongly HAD⁺ and were beginning to detach from the surface of the culture dish (Fig. 4a). The almost total lack of myotubes 24 h after influenza virus infection is clear from comparisons either with uninfected control cultures of similar age (for example, Fig. 4c); or with similar cultures infected with the lentogenic NDV, which did not cause myotube death by 24 h after infection (Fig. 4b).

These observations establish for the first time that, unlike those viruses which require cellular DNA synthesis for their replication, the RNA viruses of influenza and Newcastle disease can directly infect myotubes of cultured chicken skeletal muscle, in which the cells have terminated DNA synthesis^{9,10}. In addition, under the conditions described, it was demonstrated that, before syncytia formation, cultured muscle cells are inefficiently infected by influenza virus, based on the inability to detect synthesis of the viral haemagglutinin.

Lack of influenza replication in the prefusion muscle cells is unlikely to be an artefact of the multiplicity of infection or host-adaptation compared with NDV. Experimental conditions were identical for pre- and post-fusion cell cultures, the single exception being the state of cellular differentiation. Multiplicities of infection were similar for the influenza and Newcastle disease viruses, and the observations reported were highly reproducible using several different batches of tissue



Fig. 3 Detection of virus growth in post-fusion muscle cell cultures. Conditions were similar to Fig. 2, except that cell cultures were not infected until 40 h after plating, by which time maximum formation of myotubes had occurred. *a*, Culture infected with influenza virus and containing myotubes which were almost all HAD⁺, whereas residual single cells were HAD⁻. *b*, Cultures infected with NDV and containing HAD⁺ single cells and myotubes.

culture-grown virus, although it is not yet known whether the same behaviour holds true for other strains of influenza.

The observation that the state of cellular differentiation can



Fig. 4 Cytopathic effect of influenza virus on myotubes in cultured muscle cells. Conditions were similar to Fig. 3a and b, except that infected cultures were maintained for 24 h before testing for haemadsorption. *a*, Cultures infected with influenza virus, virtually devoid of all myotubes, but which contains an occasional HAD⁺ myotube, showing early cytopathic effect and also beginning to detach from the cell sheet. Single cells are HAD⁻. *b*, Culture infected with NDV, and containing HAD⁺ myotubes and single cells. *c*, Control culture.

affect the ability of muscle cells to support replication of an influenza virus is novel. Since orthomyxoviruses and paramyxoviruses both initiate infection by attachment to sialic acid-containing receptors on cell membranes followed by fusion of virus and cell membranes²⁰⁻²², it seems improbable that the low efficiency with which the influenza virus infected myoblasts was the result of a cell surface restriction. The different behaviour of NDVs and influenza viruses suggests rather that the restriction on influenza virus replication observed in myoblasts was related to functions in the host cell nucleus. That this restriction ceases after myotube formation might well be one result of the changes that occur in the nuclear activities of the muscle cells at the time of myoblast fusion. Further experimentation is required to justify this hypothesis; if valid, the system we have described should prove valuable in studying the role of the host cell nucleus during influenza replication, and the nature of changes in the muscle cell nucleus during differentiation.

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The reversal of methotrexate toxicity by thymidine with maintenance of antitumour effects

METHOTREXATE (MTX) is effective in the treatment of acute lymphoblastic leukaemia¹, choriocarcinoma², Burkitt's lymphoma³, and a number of other solid tumours⁴⁻⁶. An increased therapeutic index for methotrexate with delayed citrovorum factor (CF) has been reported in experimental tumour systems⁷ and in patients with head and neck cancer⁸ and osteogenic sarcoma⁹. The biological basis for this increased therapeutic index is not known, but delay in administration of citrovorum factor is a crucial feature. We have studied the effects of thymidine (TdR) on the toxicity and therapeutic effect of MTX in mice bearing leukaemia L1210. CF was included in the experiments as a positive control, and allopurinol because this drug has been reported to impair the antitumour effects of MTX⁹.

L1210 Leukaemic cells (10⁵) were injected intraperitoneally into BDF₁ male mice, and MTX, CF, TdR, or allopurinol injected intraperitoneally at least 24 h after the inoculation of leukaemic cells. The animals were weighed

Table 1 The effect of thymidine and citrovorum factor in methotrexate toxicity in normal mice

	Drug	Dose (mg kg ⁻¹)	Schedule	No. of deaths/No. of mice	Day of death
Expt 1	MTX	200	qd × 1	1/8	8
		250	qd × 1	7/8	7 ^a
		300	qd × 1	6/8	5,6 ^a
	TdR	500	tid × 4	0/8	—
	MTX TdR	200	qd × 1	1/8	6
		500	tid × 4		
	MTX TdR	250	qd × 1	1/8	6
		500	tid × 4		
	MTX TdR	250	qd × 1	5/8	6 ^a ,7
		500	tid × 4		
Expt 2	MTX	300	qd × 1	1/8	7
		400	qd × 1	4/8	7 ^a ,8
	TdR	500	tid × 3	0/8	—
	MTX TdR	300	qd × 1	0/8	—
		500	tid × 3		
	MTX TdR	400	qd × 1	0/8	—
		500	tid × 3		
	MTX CF	400	qd × 1	0/8	—
		500	bid × 3		

The first dose of CF or TdR in combination experiments was given at the same time as MTX.

qd × 1 = once.

tid × 3(4) = 3 times per day for 3(4) days.

Superscript numbers, number of animals dying.

weekly and observed for toxicity and survival. Since animals dying of drug toxicity died before the leukaemic control animals, this allowed the distinction between toxic and tumour deaths to be made⁷.

Table 1 shows that TdR (500 mg kg⁻¹ intraperitoneally three times per day for 4 d), the first dose being given at the same time as MTX, substantially reduced MTX toxicity in normal animals. Figure 1 shows that TdR similarly prevented MXT toxicity in tumour-bearing animals but did not inhibit the antitumour effects of MTX; if allopurinol was added to the MTX-TdR combination, the life of tumour-bearing animals was not prolonged. If the first TdR administration was delayed for 12 h after MTX treatment, the selective rescue effects of TdR were still observed,

and the therapeutic effect was superior to that produced by delayed CF administration. TdR pellets implanted subcutaneously also protected normal tissues from MTX toxicity although the antitumour effects were not inhibited (ref. 10 and M.H.N.T., B.L.B., and E.F., unpublished).

These experiments indicate that in mice TdR prevents MTX toxicity in normal host tissues but does not inhibit its antitumour effects. MTX-TdR seems to be superior to MTX-CF in the treatment of animals with L1210 leukaemia. The biological basis for these observations has not been established. The data suggest that MTX treatment causes thymidylate starvation in normal tissues, which can be reversed by the administration of excess TdR; and since the tumour is not rescued by thymidine, it is possible that

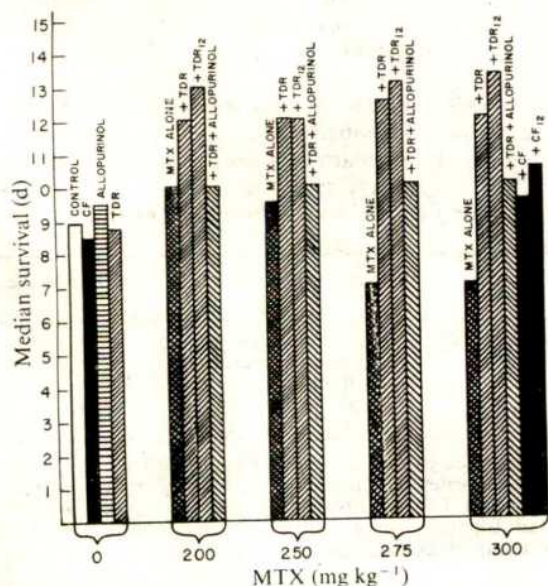


Fig. 1 The effects of MTX, TdR, CF and allopurinol in L1210 leukaemia. Histogram of median survival of control and drug-treated animals. The numbers of animals in each group was at least six, and the first administration of drug was 24 h after intraperitoneal transplantation of 10⁵ ascites cells. MTX was given as a single injection; TdR (500 mg kg⁻¹ three times per day for 3 days) was given at the same time as MTX, alone, with allopurinol, or the first dose was delayed for 12 h after MTX (TdR₁₂). Citrovorum factor (200 mg kg⁻¹) was given at the same time as MTX or the first dose was delayed for 12 h (CF₁₂). MTX caused no significant increase in survival time. MTX-TdR (simultaneous and delayed) at all doses caused significant increase in survival time over control ($P < 0.0001$). MTX-TdR and allopurinol was significantly less effective than MTX-TdR alone: at MTX 200, 250, 275 mg kg⁻¹, $P < 0.001$; at MTX 300 mg kg⁻¹, $P < 0.05$ (Mann-Whitney tests). Methotrexate sodium (4-amino-N¹⁰ methyl pteroylglutamic acid sodium), and citrovorum factor (5-formyl tetrahydrofolic acid, calcium leucovorin) were obtained from Lederle Labs, Pearl River, New York. Allopurinol (4-hydroxy-pyrazolo (3,4-d) pyrimidine) was obtained from Burroughs Wellcome, London and TdR from Nutritional Biochemical Corp.

MTX causes additional disturbances in tumour tissues. Hryniuk¹¹ has reported that MTX causes death in rapidly proliferating L5178Y cells in culture as a result of a lack of purine. Grindey and Moran⁹ observed that allopurinol, which elevates plasma and tissue purine levels, reduced the antitumour effects of MTX in mice with L1210 leukaemia. All these data indicate that MTX treatment may cause purine starvation in L1210 tumour cells *in vivo*. The retention of non-dihydrofolate reductase protein by MTX-Sepharose indicates that MTX may have multiple sites of action¹². It is known, however, that reduced folates are 1 carbon donors in thymidylate and *de novo* purine biosynthesis. As bone marrow cells utilise predominantly pre-formed purines synthesised by the liver¹³, for nucleic acid synthesis it seems possible that TdR may rescue normal proliferating host tissues, which do not rely on *de novo* purine biosynthesis, but not rescue tumour cells in which *de novo* biosynthesis may be the major source of purines.

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Microsomal enzyme induction by methadone and its implications on tolerance to methadone lethality

MANY investigators have sought to explain narcotic tolerance as a result of increased metabolic inactivation of the drug. No evidence for disposition tolerance has yet been reported, however¹⁻⁶. On the contrary, tolerance to most narcotic effects seems to be adaptive or cellular tolerance, that is a decrease in pharmacological effects even when enough drug is in contact with target cells in the brain to produce marked effects in naive animals⁷. We wish to report, however, that metabolic inactivation of methadone, a widely used synthetic narcotic, does increase, with a resulting tolerance to its toxic properties.

Subcutaneous or oral administration of methadone has been shown to prevent one sign of narcotic withdrawal in

mice tolerant to and dependent on morphine after implantation of a morphine pellet for 3 d (ref. 8 and L. M., unpublished). After 6 d of oral methadone treatment (100 mg kg⁻¹ of the hydrochloride salt), these mice display tolerance to the lethal effects of methadone, but not to those of morphine (Table 1). This observation suggests the existence of disposition tolerance to methadone, as an increased LD₅₀ for morphine, as well as methadone would have been expected had cellular tolerance developed in these animals.

The liver seems to be the chief site of metabolism for methadone⁹, which is a substrate for the rat and rabbit hepatic microsomal N-demethylase enzymes¹⁰. We therefore examined the activity of N-demethylase in the livers of mice maintained on the same regimen as that described above. The activity of N-demethylase was assayed in the hepatic 12,000g supernatant fraction¹¹ 6 d after removal of the morphine pellet. Ten micromoles of D,L-methadone were used as substrate. The 6-d oral administration of methadone brought about a nearly twofold increase in the activity of the enzyme. This increase in enzyme activity may account for the elevation of the methadone LD₅₀ which was determined in identically treated animals. Since N-demethylation plays only a minor role in the inactivation of morphine⁷, the fact that the LD₅₀ for morphine was unchanged is not surprising.

To demonstrate that the elevation in the methadone LD₅₀ was the result of increased N-demethylase activity following methadone administration and not residual cellular tolerance brought about by the morphine pellet treatment, we gave naive mice phenobarbital intraperitoneally (50 mg kg⁻¹ d⁻¹), which increases the activity of a variety of microsomal enzymes¹². After 3 d of treatment, a nearly sixfold increase in N-demethylase activity occurred (Table 2). There was a concomitant fourfold increase in the oral LD₅₀ for methadone. A less dramatic but significant increase of 55% in the intraperitoneal methadone LD₅₀ was also noted. In contrast, the intraperitoneal morphine LD₅₀ was not significantly affected by the barbiturate treatment. Since these mice had never been exposed to narcotics, it seems that a change in disposition of methadone did occur.

The increased activity of N-demethylase observed as a result of methadone treatment is not a consequence of the morphine pellet treatment since morphine treatment has been shown to lower N-demethylase activity significantly². To demonstrate the ability of methadone to increase N-demethylase activity, naive mice were given oral doses of methadone (50 mg kg⁻¹) daily. After 24 h, a 50% increase in the activity of N-demethylase was noted. By day 6, the activity was increased nearly twofold (compare 0.770 ± 0.124 with 1.419 ± 0.124 μmol of formaldehyde per 30 min per liver).

Table 3 demonstrates the similarities between the effects of methadone and phenobarbital on liver protein content and liver weights. Both treatments caused a significant elevation in the liver weights and in the protein of the 12,000g supernatant fraction and of the microsomal fraction of the liver.

Table 1 Effects of repeated methadone treatment on methadone and morphine lethality and activity of liver N-demethylase in mice rendered tolerant by implantation of a morphine pellet

Treatment	Methadone LD ₅₀ ± s.e. (mg kg ⁻¹ , p.o.)	Morphine LD ₅₀ ± s.e. (mg kg ⁻¹ , i.p.)	N-demethylase activity (μmol formaldehyde/ 30 min/liver ± s.e.)
Water	120 ± 4 (24)	404 ± 27 (24)	0.770 ± 0.124 (11)
Methadone	198 ± 21* (24)	407 ± 34 (24)	1.42 ± 0.12* (11)

Mice were implanted with 75 mg morphine base pellets for 3 d (ref 8). Treated animals were given 100 mg kg⁻¹ of D, L-methadone HCl in water (5 mg ml⁻¹) orally once a day for 6 d after removal of the pellet. Controls were given equivalent volume of water. All determinations were made approximately 5 mg of microsomal protein per assay from the 12,000g supernatant fraction of mouse livers. The assay procedure of Fouts¹¹, using 10 μmol (2 mM) of D, L-methadone HCl as substrate, was used. Numbers in parentheses indicate number of animals in each group.

* Significantly different from controls, *P* < 0.05.

i.p., Intraperitoneally; p.o., orally.

Table 2 Effect of phenobarbital treatment on methadone and morphine lethality and activity of liver N-demethylase in naive mice

Treatment	Methadone LD ₅₀ ± s.e. (mg kg ⁻¹)	Morphine LD ₅₀ ± s.e. (mg kg ⁻¹)	N-demethylase activity (μmol formaldehyde/30 min/liver ± s.e.)
Saline	84.0 ± 4.6 (24), p.o. 40.0 ± 2.8 (24), i.p.	480 ± 44 (24)	0.598 ± 0.026 (5)
Phenobarbital	304 ± 42* (24), p.o. 62.0 ± 2.6* (24), i.p.	547 ± 23 (24)	3.58 ± 0.23* (5)

Mice were injected intraperitoneally with 50 mg kg⁻¹ phenobarbital in saline (5 mg ml⁻¹) once a day for 3 d. Controls were injected with saline. All determinations were made 24 h after the last injection using the same procedures as in Table 1. Numbers in parentheses indicate number of animals in each group.

* Significantly different from controls, $P < 0.05$.

The increases in microsomal protein and N-demethylase activity after methadone seem to be typical of the phenobarbital type of induction of microsomal enzymes¹².

The principal metabolites of methadone are 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (M₁) and 2-ethyl-5-methyl-3,3-diphenyl-1-pyrroline (M₂)^{13,14}. Both are formed by N-demethylation of methadone and non-enzymatic cyclisation and, in the case of M₂, further N-demethylation¹⁵. These metabolites have been isolated from rat bile^{14,16} and urine¹⁶, as well as from human plasma^{17,18}, bile^{14,17} and urine^{13-15, 17,19}. They have no demonstrated analgesic activity¹⁴ and, therefore, probably play no role in the prevention of the narcotic abstinence syndrome. Thus, the increase in the activity of N-demethylase following repeated administration of methadone should increase the rate of

drug (50 mg kg⁻¹). Subcutaneous administration of methadone brought about a much smaller decrease in this half-life.

These observations may have clinical implications for methadone maintenance in which doses are often greater than one-half the minimal lethal dose in non-tolerant subjects. Rapid inhibition of microsomal metabolism can follow after exposure to a number of drugs and environmental agents^{26,27}. Morphine and heroin, for example, are abused by subjects on methadone maintenance and are effective microsomal enzyme depletors^{2,28}. Furthermore, the occurrence of microsomal enzyme inhibition is compounded when methadone is taken orally, a route which is associated with a slow onset and prolonged duration of drug action. Thus, toxic sequelae may not develop until the subject has

Table 3 Effects of phenobarbital and methadone on liver weights and protein in liver fractions

Treatment	Liver weight ± s.e. (g)	Protein in 12,000g supernatant fraction of liver ± s.e. (mg)	Protein in microsomal fraction of liver ± s.e. (mg)
Naive mice			
Saline (5)	1.42 ± 0.06	159 ± 7	62.6 ± 2.8
Phenobarbital (5)	1.68 ± 0.08*	203 ± 11*	85.9 ± 4.3*
Mice implanted with morphine pellet			
Water (10)	1.37 ± 0.06	109 ± 8	50.4 ± 3.6
Methadone (14)	1.69 ± 0.06*	127 ± 6*	60.4 ± 2.7*

Naive mice were injected with phenobarbital or saline according to the procedure in Table 2. Mice implanted with 75 mg morphine base pellets were given either D,L-methadone HCl or water orally for 6 d according to the procedure in Table 1. All determinations were made 24 h after the last dose. The microsomal fraction was obtained by centrifuging 1-ml aliquots of the 12,000g supernatant fraction at 105,000g for 1 h. Proteins were determined by the method of Lowry *et al.*³⁰. No significant differences were noted in the body weight data. Numbers in parentheses indicate number of animals in each group.

* Significantly different from controls, $P < 0.05$.

inactivation of the narcotic and contribute to the development of tolerance to some methadone effects.

There is circumstantial evidence for the role of metabolism in the termination of the pharmacological actions of methadone. Basic metabolites of methadone are significantly elevated in the faeces of rats after repeated methadone administration²⁰. These compounds are probably M₁ and M₂ since both have a higher pK_a than methadone²¹ and represent the greatest portion of methadone metabolites^{14,15}. More recently, pretreatment with methadone for 15 d was shown to increase the biliary excretion of M₁ by 50% and advance its peak excretion time¹⁶. Since biliary excretion accounts for the majority of these compounds in the faeces²², these two observations support the possibility that increased activity of microsomal enzymes is the consequence of the repeated administration of methadone.

Alvares and Kappas²³ showed that phenobarbital pretreatment of rats results in an enhancement of demethylation of methadone *in vitro* by liver microsomal preparations and that the analgesic effect of methadone decreases concomitantly. No increased N-demethylase activity was demonstrated following chronic administration of methadone (20 mg kg⁻¹, intraperitoneally), however. Peters²⁴ was also unable to demonstrate enhanced demethylation after daily intraperitoneal injections of methadone (5 mg kg⁻¹) in rats. In contrast, Misra *et al.*²⁵, using an oral rather than a parenteral route, observed that the biological half-life of methadone in the plasma and brain of rats was reduced more than fourfold after repeated administration of the

left the methadone maintenance centre for the day.

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Increased neonatal mortality in offspring of male rats treated with methadone or morphine before mating

METHADONE ((\pm)-4, 4-diphenyl-6-dimethylamino-3-heptanone hydrochloride) is a potent (addictive) analgesic with pharmacological effects which are qualitatively similar to those of morphine, although it is far more effective than morphine when given orally. Its ability to diminish the severity of the abstinence syndrome resulting from heroin withdrawal led to the introduction of methadone in the chemotherapy of narcotic addiction¹⁻³. Little is known, however, about its long term toxicity effects. We report that treatment of male rats with either (\pm)-methadone HCl (METH) or morphine sulphate (MS), given orally, for 24 h before mating to untreated females increases the neonatal (21-d) mortality of their offspring.

Sixty naive female Charles River (CD) albino rats (which had not been previously treated with the drug) were mated to 26 male rats which had received 13, 18 or 38 mg kg⁻¹ d⁻¹ METH during

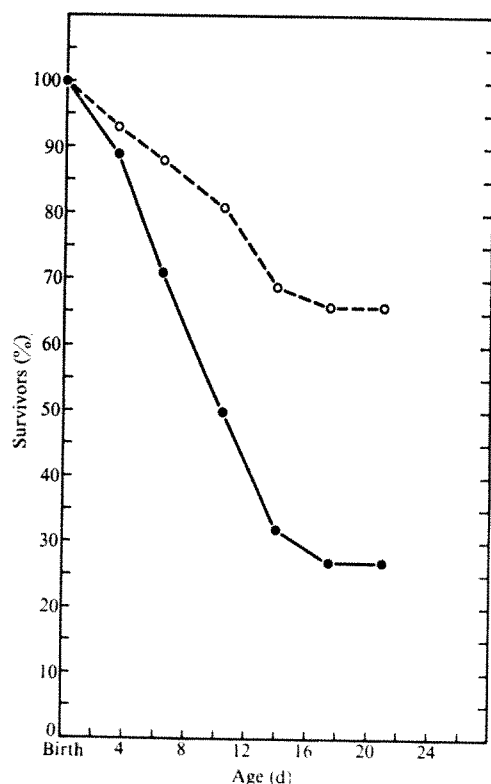


Fig. 1 Neonatal (21-d) survival of 166 live offspring sired by male rats pretreated with MS (○) and 145 live offspring sired by male rats pretreated with METH (●). Twenty-six per cent of the offspring sired by METH pretreated males and 66% of the offspring sired by the MS pretreated males survived. Of the live offspring of untreated males 90-95% survived for 21 d.

the previous 24 h. Another 60 naive female rats were mated to 26 other male rats similarly treated with 22, 47 or 55 mg kg⁻¹ d⁻¹ MS. Females were exposed to treated males for 7 consecutive days.

Figure 1 shows the marked difference in the 21-day mortality of the offspring. Those from MS-treated males (six fostered and ten unfostered litters) had 57 deaths out of 166 live births (34% mortality), whereas those from METH-treated males (seven fostered and seven unfostered litters) had 107 deaths out of 145 live births (74% mortality). This difference is very significant ($\chi^2 = 46.77$, $P < 0.001$). The METH offspring ($n = 145$) died at the rate of 8 per day from days 4 to 14, whereas the MS offspring ($n = 166$) died at a rate as high as 6 per day only between days 11 and 14, suggesting that a different lethal mechanism is operating. Overall mortality in both groups is considerably greater than the 5.9% rate in 1,645 offspring resulting from matings of untreated males and females, and reared by untreated foster mothers^{4,5}.

We also investigated the effects of pretreating male rats for 24 h with METH given 0-24 h or 25-48 h before mating with naive females ($n = 12$ in each group). Males were first placed in a cage and provided with a 5% sucrose drinking water

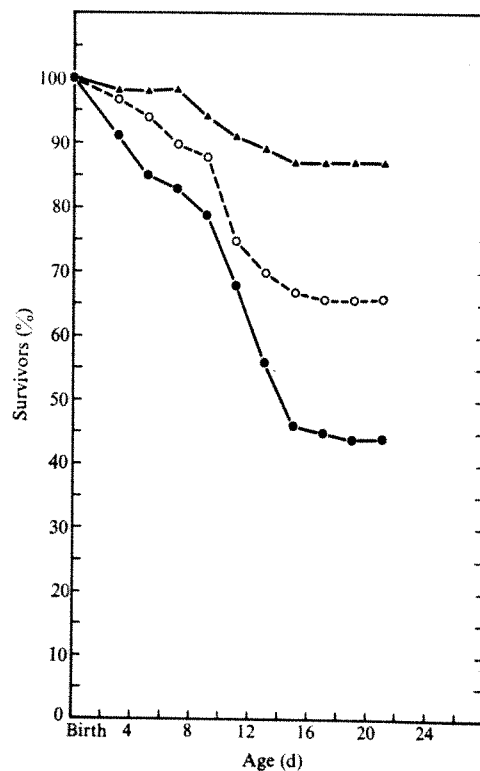


Fig. 2 The 21-d survival of the offspring of naive females mated with male rats pretreated with either no drug (▲) or with low-dose METH (○, 25 mg kg⁻¹ d⁻¹) or high-dose METH (●, 52 mg kg⁻¹ d⁻¹) during the 24-h period before mating. These three mortality rates differ significantly ($P < 0.01$) from each other.

solution containing 0.67 mg ml⁻¹ or 0.067 mg ml⁻¹ METH. The 'high-dose' METH males received 52 mg kg⁻¹ d⁻¹, the 'low-dose' METH males 25 mg kg⁻¹ d⁻¹. A third group received sucrose solution alone. Each male remained in this cage with four females for 24 h. (The offspring of these females are not part of the present study.) Males were then moved to a second cage provided with drinking water with neither drug nor sucrose, containing four drug-naive females, the offspring of which are identified as the '0-24 h' offspring, as the males had received METH in that period prior to siring these offspring. After 24 h with these females each male was then moved to a third cage provided with pure drinking water and containing

four more naive females, the offspring of which are identified as the '25-48 h' offspring. After 24 h with these females each male was then returned to the initial cage containing the drinking water with 5% sucrose solution (with or without METH) and the cycle repeated. Each naive female was exposed to males for 6 consecutive days.

The neonatal (21-day) mortality of the offspring of the three groups of males is shown in Fig 2. In 21 days, 13% of 132 offspring sired by control males died, whereas 56% of 100 offspring sired by high-dose METH males died (difference very significant $\chi^2 = 47.08$, $P < 0.001$) and 34% of 100 progeny of low-dose METH males died (difference from controls significant $\chi^2 = 13.59$, $P < 0.01$, and from high-dose of METH progeny $\chi^2 = 8.91$, $P < 0.01$). The neonatal mortality of 244 offspring of males which had received METH 25-48 h before mating was only 25%. This did not differ significantly ($\chi^2 = 3.41$, $P > 0.05$) from the 21-day mortality of their controls (17%). Of the ten litters sired by control males only one had a death rate in excess of 30% (Fig 3), whereas of the eight litters sired by the high-dose METH males, six litters lost 40% or more of the offspring born alive. The low-dose methadone litters had a smaller death rate than the high-dose METH offspring. These data demonstrate that the increased mortality of the METH-sired offspring is not simply a consequence of the deaths of the offspring of a small proportion of the males, but of an increased mortality in virtually all litters.

Pretreatment of male rats with METH before mating thus produces a significant increase in neonatal (21-day) mortality of the offspring of the METH-treated males and naive females when compared to control matings. Our data show a dose-response relationship for this phenomenon as well as evidence

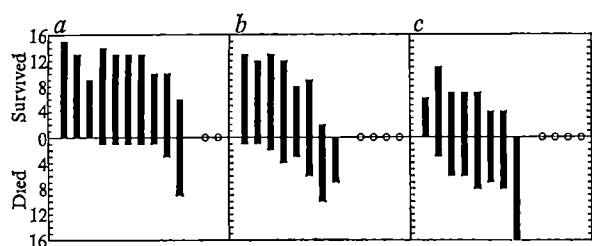


Fig 3 Survival and death in each litter of offspring born to females mated to (a) controls and males receiving (b) low- and (c) high-dose METH 0-24 h before mating. The vertical black bars show the survival or death of the live offspring from each litter. \circ , Females which did not deliver offspring.

that the lethal effect is seen primarily in offspring sired during the first 24 h following administration of METH to the male. Morphine produced a similar but less marked effect. An urgent problem in medicine is the fact that 1-3% of all infants born have one or more unexpected congenital anomalies which cannot be explained by classical teratology. Our preliminary results suggest that it is necessary to investigate not only the drugs which were administered to the mother before and during pregnancy, but also those drugs used by the father. This approach may contain the answers to some of the unexplained problems of childhood growth and development.

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Physiology of visual cells in mouse superior colliculus and correlation with somatosensory and auditory input

THE two main targets of the mammalian optic nerve fibres are the lateral geniculate body and the superior colliculus (optic tectum). From studies with various techniques, and in several mammalian species including the cat¹⁻⁵, monkey⁶⁻⁸, rabbit¹⁰⁻¹², rat¹³, and ground squirrel¹⁴ three major functions of the superior colliculus have been described. In the superficial layers the visual input is processed in a specific way; in deep layers several sense modalities, chiefly visual, auditory and somatosensory, are brought together, stimulation of the tectum results in an orienting of the animal's eyes, head or body towards a location corresponding topographically to the part of the tectum stimulated.

Gordon¹ has shown that for a given location in the cat tectum there is a good correlation between the positions of visual receptive fields and the directions from which maximal auditory responses are evoked. She also found a correlation between preferred directions of stimulus movement in the two modalities. The agreement between visual and somatosensory cells was much looser, although visual receptive fields near the vertical midline were correlated with tactile fields on the face, and temporal visual fields, with tactile fields on the body or legs. She rarely observed responses from whiskers. Cats move their eyes and especially their heads extensively, however, and a very close relationship between retinotopic and somatosensory maps was perhaps not to be expected.

We have studied the superior colliculus in the mouse, a small compact animal with very little eye movement and relatively little head movement, a mouse rather turns its whole body towards an interesting object. The major part of the mouse's visual field is crossed by an elaborately developed somatosensory organ, the vibrissae. Whisker movement in the mouse is very rapid, but the excursions are small, so that the whiskers bear a relatively constant relationship to the visual field. One should therefore not be surprised to find a close topographical relationship between the tectal projections from retina and whiskers.

In our study, ten mice of the C57BL/6J strain were used. Throughout the experiment a mouse was kept under light anaesthesia (pentobarbital and chlorprothixene) and was neither paralysed nor artificially respirated (For exact procedures, see Dräger¹⁵). The head was held fixed by means of a small metal block glued to the skull, thus leaving the ears free. Visual receptive fields were mapped on a translucent tangent screen placed at a distance of 10.5 cm from the mouse. Electrolytically polished tungsten electrodes were used for recording. Electrode tracks, most of which were perpendicular to the tectal surface, were marked by several small electrolytic lesions (2 μ A for 2 s) and reconstructed histologically. A total of 323 recordings were made in 48 penetrations. Of these records, 145 were probably from tectal cells. The remaining 178 recordings, from unit clusters or poorly resolved units, were useful for purposes such as topographical mapping of the tectal surface.

Cells in the superficial layers had small visual receptive fields (average diameter 9°) whose locations in the visual field varied according to a topographical map similar to that described for other vertebrates¹⁶: the nasal visual field projected anteriorly and the upper visual field medially. These cells responded only to visual stimulation, with best responses to a slowly moving small spot of any shape. One-quarter of the cells preferred one direction of movement, for most cells, the preferred direction was upward. Deeper in the tectum most visual cells had very different receptive-field properties, with large fields (20°-60°), sluggish and transient responses to small moving objects, little or no response to large stimuli, and often a directional selectivity with preference for upward movement. Characteristically these deeper-layer cells tended

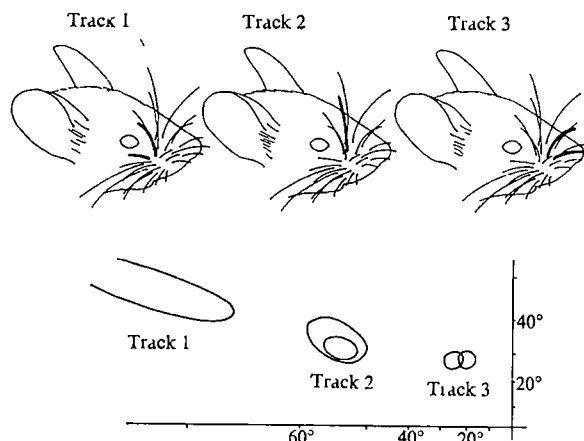


Fig. 1 Correlation between somatosensory and visual receptive fields recorded in three successive electrode penetrations. Lower part of diagram shows visual receptive fields recorded in superficial tectal layers and mapped on tangent screen. Mouse was facing the tangent screen at right angles, 10.5 cm behind it. Origin of coordinate axes marks intersection with the long axis of the mouse. Upper part of the diagram indicates whiskers giving strongest responses in the same electrode tracks at a deeper level, these whiskers are drawn as thicker lines.

to habituate if the same stimulus was repeated, but fired again if the spot or object was moved through a different part of the field. Similar visual receptive field properties in the superior colliculus have been described in other species^{5,6,10,12,13}.

At a slightly deeper level, in the stratum griseum mediale, cells responding to somatosensory or auditory stimulation were first encountered intermixed with visual cells, and with cells responding to two (or rarely to all three) modalities. Still deeper in the tectum, over a distance of several hundred μm , somatosensory or auditory modalities tended to take over completely until, in some penetrations, the electrode again entered an area of mixed responses. There was no segregation of somatosensory and auditory cells into sub-layers, cells responding to a given modality seemed rather to be arranged in clusters with some intermixing at the boundaries between clusters.

Somatosensory responses were obtained about twice as frequently as auditory. In most penetrations the tactile receptive fields were located on one or a few whiskers. Gently tapping one whisker evoked a short burst of spikes with little difference in response as the direction of hair deflection was varied.

The most striking feature was a very consistent relationship in any one electrode track between the location of visual receptive fields and that of somatosensory receptive fields. A series of three electrode tracks in one experiment is illustrated in Fig. 1. The axes in this illustration represent the horizon and the vertical midline of the mouse, drawn on the tangent screen so as to cross at the extended longitudinal axis of the mouse. Numbers on the axes mark degrees of eccentricity. Visual receptive fields in the three tracks are outlined on the screen. In the upper part of the figure, the whiskers giving the strongest responses from cells deeper in the tectum are drawn more heavily than the others. In the first track there was a clear correlation between temporal visual fields and posterior whiskers, moving nasally in the visual field in tracks 2 and 3 was accompanied by movement to more anterior whiskers. In other series of penetrations a similar correlation was seen between visual receptive-field positions and the whiskers that evoked the best responses at deeper levels in the tectum. If the visual receptive fields were progressively lower in a set of tracks, the whisker fields likewise moved down. By drawing the projections of the whiskers through the eye on to the tangent screen, we could show that for a given perpendicular penetration the visual receptive fields were crossed by just the whiskers that evoked maximal responses from the somatosensory cells

deeper in the tectum. This correlation held not only for neighbouring visual and somatosensory cells, but also for single cells driven by both modalities.

In parts of the tectum in which visual receptive fields were far peripheral, and no whiskers lay in the way, somatosensory responses at deep levels were evoked from other parts of the body. Visual fields far down, where the mouse might see its own paw, were associated with tactile fields on the dorsum of the paw. Far temporal visual fields were correlated with somatosensory fields on the flank, shoulder and ear, here an upward movement in the visual field was paralleled by upward movement in the somatosensory map, from the flank to the tip of the ear.

Reactions to auditory stimulation were found less frequently than to tactile stimuli and they tended to be less well localised. Auditory responses could be evoked by complex sounds rich in high frequencies like clicks or crackling noises. Usually only sounds generated from a direction contralateral to the colliculus were effective in driving auditory cells. In eight out of thirteen cells the auditory receptive field in the horizontal plane was restricted to an angle of about 50° – 150° , which included the visual and somatosensory receptive field of cells recorded simultaneously or in the close vicinity.

The mouse superior colliculus thus contains three topographical maps, superimposed and roughly in register, of the surroundings as observed through visual, somatosensory and auditory modalities. We assume that this system serves to orient the animal to an interesting stimulus whatever the sense modality.

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Conductance of channels opened by acetylcholine-like drugs in muscle end-plate

MOST recent views of the way in which acetylcholine (ACh) can cause ion channels to open postulate that the channel can exist only in one or other of two distinct conformations, open or shut^{1–4}. In their simplest form, these theories predict that a channel, once it is open, will have the same conductance whichever drug caused it to open. We have estimated average single channel conductances for four cholinomimetic agonists, and find this prediction is not confirmed.

The mean single-channel conductance, and mean lifetime of the open state, have been estimated at the voltage-clamped end-plate of the frog (summer *Rana pipiens*) cutaneous pectoris muscle. Methods similar to those of Anderson and Stevens⁵, were used with the addition of Normarski interference optics,

which make the end-plate clearly visible. The drugs shown in Fig 1 were applied iontophoretically to the end-plate, and samples of the end-plate current fluctuations taken at $1,024 \text{ s}^{-1}$. The mean single-channel conductance, γ , was estimated from the total variance, s_I^2 , of the current fluctuations, as $\gamma = s_I^2/m_I(V - V_{eq})$, where m_I = mean end-plate current induced by the drug, V = membrane potential and V_{eq} = equilibrium potential. This method assumes that the fluctuations represent the random opening and closing of channels, and that no appreciable contribution to s_I^2 occurs outside the frequency range examined in our experiments (1–500 Hz).

Measurements in both normal and in glycerol-pretreated muscle fibres showed that V_{eq} was the same (near 0 mV), within a few mV, for all the drugs in Fig 1. Measurements on normal fibres were obtained by depolarising in small steps of a few mV, spaced by about 10 s, as the contraction threshold (about –55 mV) was approached, until about –30 mV was reached at which point the excitation-contraction mechanism was inactivated. The variance was estimated from the variance of the digitised noise record, and as

$$s_I^2 = \int_{-\infty}^{\infty} S(f) df = \int_0^{\infty} G(f) df$$

where $S(f)$ is the two-sided, and $G(f) = 2S(f)$ the one-sided spectral density computed from the fluctuations. These methods gave very similar results.

The results are shown in Table 1. The means for all experiments are given, because the results were reproducible from day to day. For example, three separate experiments with suberyllcholine (SubCh) gave $\gamma = 28.4 \pm 1.6$, 28.2 ± 2.2 , and $29.2 \pm 2.4 \text{ pmho}$.

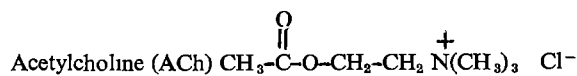
It is clear from these results that the mean single-channel conductances, γ , are not the same for all drugs, but that they vary over an approximately twofold range for the drugs tested. In all seven experiments in which SubCh and ACh were tested on the same cell, it was found that SubCh had a larger γ than ACh, the mean ratio being 1.19 ± 0.04 . SubCh also had a longer mean open lifetime than ACh, by a factor of 1.7 (Table 1), the same as the factor found by Katz and Miledi⁷. The agonists 3-phenylpropyltrimethylammonium (PPTMA) and 3-(*m*-hydroxyphenyl)propyltrimethyl ammonium (HPTMA), which are full agonists as judged by their ability to cause contraction of the frog rectus abdominis muscle⁸, have a substantially smaller γ than either ACh or SubCh.

Katz and Miledi⁷ have already shown that the mean length of time for which the ion channel stays open may differ over an approximately tenfold range for different drugs, at the frog muscle end-plate. Contrary to the predictions of the simple two-state theories, it seems that differences in agonist action that can produce a tenfold difference in the mean time that a channel remains open, can also influence the mean conductance of the open channel. For the drugs tested at least, γ varies much less than the mean open-channel lifetime, τ , from drug to drug. It is also intriguing that a large single channel conductance seems to accompany a large open lifetime, but not enough drugs have been tested yet to allow any generalisation to be made.

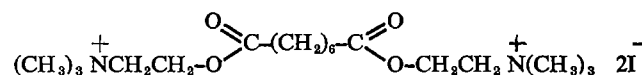
Table 1 Values of mean single-channel conductance (γ) computed from integrated spectral densities for current fluctuations, and values of mean open-channel lifetime, τ , at 10–15°C and a membrane potential of between –60 and –80 mV, computed as $1/(2\pi f_c)$ where f_c = half-power frequency

	γ (pmho)	τ (ms)
SubCh	28.6 ± 1.0 (22)	5.6 ± 0.3 (8)
ACh	25.0 ± 0.9 (8)	3.2 ± 0.3 (6)
HPTMA	18.8 ± 0.8 (19)	1.0 ± 0.06 (13)
PPTMA	12.8 ± 1.1 (7)	0.83 ± 0.02 (3)

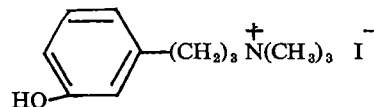
The frog Ringer solution contained tetrodotoxin (100 nM). All results are on normal (not glycerol-treated) fibres. The table gives the mean of values on all cells \pm s.e., with the number of determinations in parentheses.



Suberyllcholine (SubCh)



3-(*m*-hydroxyphenyl) propyltrimethyl ammonium (HPTMA)



3-phenylpropyltrimethyl ammonium (PPTMA)

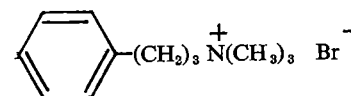


Fig. 1 Drugs applied iontophoretically to *Rana* muscle end-plate

The spectra of current fluctuations produced by ACh and SubCh could be fitted well with a single time constant ' $1/f$ ' model^{1,5}. The mean single channel conductance, estimated from the zero frequency asymptote of spectral density⁶, as $\gamma = S(0)/2m_I(V - V_{eq})\tau$, agreed well with estimates of γ from the noise variance in the case of these drugs. The spectra of the other two agonists, however, had a component at low frequency, in addition to the major high frequency component. The sum of two single time-constant spectra was found to fit these results. The 'mean open lifetimes' in Table 1 were derived from the high frequency component, but whether or not they estimate accurately the lifetime of an actual species depends on the explanation for the two components that is adopted. It is possible that several species of channel exist with different mean open times, corresponding, conceivably, with the binding of different numbers of agonist molecules. But it can be shown quite simply that if the channels all have the same conductance when they are open, then the value of γ calculated from the noise variance should be this conductance, regardless of how the channels are distributed among the species of different mean open lifetimes⁶.

Two classes of explanation can be imagined for the observation that mean single channel conductances are not the same for all drugs. It could be that the channel can exist in several distinct open conformations, each of which has a different conductance, and that different drugs favour different conformations. On the other hand, it could be that the open channel has essentially the same conformation for all drugs, but individual drugs have, for example, somewhat different effects on local field strength or local pH so that each drug affects the pK of ionisable groups, or access of ions to the channel, in a slightly different way. It has been reported⁹ that reduction of the ACh receptor with dithiothreitol changes ACh voltage fluctuations in a way which suggests that the primary change is a lowered single-channel conductance.

Our conclusions depend, of course, on the correct estimation of γ . It is conceivable, for example, that current fluctuations actually reflect receptor occupancy by the drug⁵, and that the channel oscillates more rapidly than we can observe between open and shut states while it is occupied. In this case our estimates of γ would be only a lower limit of the true open channel conductance.

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Reversible spectral change of squid retinochrome by salts

THE cephalopod retina contains two kinds of photosensitive pigments, rhodopsin and retinochrome. Retinochrome was first found in the inner segment of squid visual cells¹. More recently it was reported that the outer segment might also contain retinochrome together with rhodopsin². The chromophore of retinochrome, all-*trans* retinal, is predominantly converted into the 11-*cis* form by illumination. It has been suggested that retinochrome supplies 11-*cis* retinal for the regeneration of cephalopod rhodopsin in the dark, by acting as an isomerase¹. The validity of the mechanism, however has not yet been established *in vitro* or *in situ*.

The chemical behaviour of retinochrome has been studied and compared with that of rhodopsin^{1,3}. Retinochrome has an absorption spectrum that depends on pH, and is sensitive to hydroxylamine and NaBH₄, while rhodopsin has an absorption spectrum independent of pH and is not affected by those reagents. This suggests that the link between chromophore and protein in retinochrome is more susceptible to the environment than in rhodopsin. On the other hand, evidence from circular dichroism measurements indicates that the chromophore of retinochrome is fixed to the protein part so that its rotation is restricted as in rhodopsin^{2,3}. Here we show that the absorption maximum of retinochrome is shifted to the shorter wavelength with increasing salt concentration.

Eyes were enucleated from fresh squids (*Todarodes pacificus*) under dim white light, frozen immediately, and stored at -20° C until used. For each preparation, we used about 40 eyes. They were thawed gradually at room temperature and bisected, after which the hemispheres containing the retinae were shaken in phosphate buffer (1/15 M, pH 6.8) in order to detach the outer segments of the visual cells and free the retinae

Fig. 1 Absorption spectra of retinochrome at pH 5.4 and 13° C. Curve 1 is the absorption spectrum of desalted retinochrome—digitonin solution. Next, solid NaCl (60 mg) was added to the solution (1 ml) and the pH was adjusted to 5.4 by addition of 0.6 μl 1 N NaOH (curve 2). Curve 3 is the spectrum of the sample after desalting by dialysis.

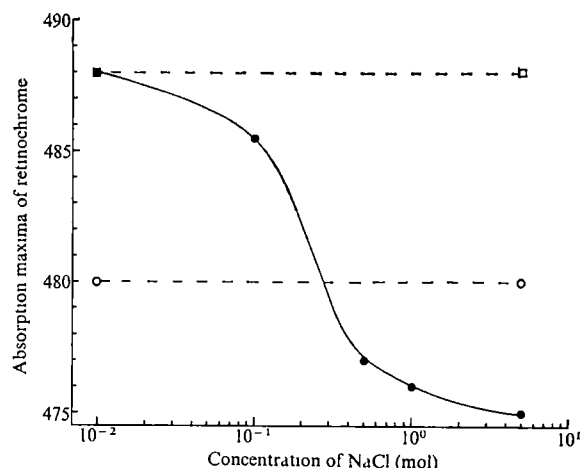
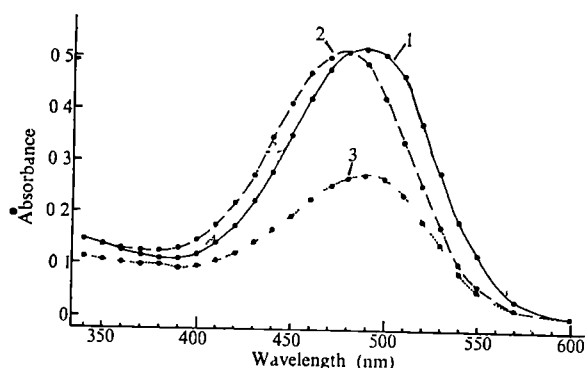


Fig. 2 Absorption maxima of retinochrome (●), rhodopsin (○) and acid-metarhodopsin (□) in various concentrations of NaCl at pH 5.4. The salt concentration of the samples was increased by addition of solid NaCl. When the sample became turbid by addition of NaCl, the absorption maximum was determined after centrifugation for 1 h at 28,000g at 4° C. Acid-metarhodopsin was prepared by converting rhodopsin to alkaline metarhodopsin by exposure to yellow light ($\lambda > 520$ nm) at pH 10, and then lowering the pH to 5.4.

containing the retinochrome. From the outer segment we extracted rhodopsin according to a method described previously². When retinochrome had been extracted from the outer segments together with rhodopsin, the two were separated by the chromatographic method described previously². Retinochrome was prepared from the outer segment-free retinae¹.

Ommochrome was eliminated from the extracts by passing them through a DEAE cellulose column (1 cm in diameter and 5 cm in height). The extract was placed on top of a column equilibrated with 0.02 M phosphate buffer (pH 6.8) and eluted with 0.02 M phosphate buffer containing 0.1% digitonin. As ommochrome was strongly adsorbed at the top of the column, eluates were free from ommochrome. The eluates were gathered, concentrated by a Sartorius membrane filter and then desalted by passage through a Sephadex G-25 column (1 cm in diameter and 20 cm in height), which was equilibrated with non-ionic 0.1% aqueous digitonin. We used the desalted retinochrome and rhodopsin samples to investigate the effect of salt on retinochrome and rhodopsin. Absorption spectra were measured with a 124 type Hitachi spectrophotometer at 13° C. Ionic strength was monitored with a conductivity meter (CD-35 M II, MS K1K1).

Figure 1 shows the absorption spectrum of retinochrome from 340–600 nm at pH 5.4. Retinochrome has an absorption peak at 488 nm in desalted digitonin solution (curve 1). When solid sodium chloride was added to this sample to a final concentration of 1 M, the absorption maximum was shifted to 478 nm (curve 2). At this salt concentration, no further change of the absorption spectrum was observed for at least 8 h at 13° C. When the sample was desalted by dialysis with a Sartorius membrane filter against distilled water for 5 h at 5° C, it again exhibited an absorption spectrum with the peak at 488 nm (curve 3). The absorbance at 488 nm was reduced to about half the initial value, because of the dilution of the sample during dialysis. This result shows that the change in the λ_{max} of retinochrome by salt is reversible.

Figure 2 shows the effects of salt concentration on the λ_{max} of retinochrome, rhodopsin and acid-metarhodopsin at pH 5.4. The λ_{max} of retinochrome shifted considerably at concentrations between 0.1 M and 0.5 M NaCl, and was at 475 nm in 5 M NaCl, while no shift of λ_{max} of rhodopsin and acid-metarhodopsin was observed. We further investigated the effect of various salts (NaCl, KCl, LiCl, MgCl₂ and CaCl₂) on the λ_{max} of retinochrome. All the salts so far examined caused the shift of the λ_{max} from 488 nm to a limit of 475 nm at pH 5.4.

with increasing concentration. The concentration necessary for a 6-nm shift, about a half the maximum, was 0.03 M for CaCl_2 and MgCl_2 , 0.2 M for KCl and LiCl , and 0.4 M for NaCl .

Next, we examined the salt effect on the absorption spectrum of retinochrome in alkaline solution. When retinochrome was brought from pH 5.4 to pH 9.5 in desalted solutions, its λ_{max} shifted from 488 nm to 495 nm. At the same time, the absorbance at 495 nm became smaller than the initial absorbance at 488 nm and another peak appeared at 370 nm, as already reported^{1,2}. When solid sodium chloride was added to the alkaline solution to a final concentration of 1 M, no shift of either of the absorption peaks at 495 nm and 370 nm was observed. Figure 3 shows the effect of pH on λ_{max} of retinochrome in digitonin solutions containing 0.01 M and 1 M NaCl . The λ_{max} of retinochrome gradually changed from 484 nm at pH 4.5 to 495 nm at pH 9.3 in 0.01 M solution. In 1 M NaCl the λ_{max} was 476 nm at pH 4–5 and shifted to longer wavelengths above pH 6, reaching a limit of 495 nm at pH 9.3. This result indicates that the salt affects the pH dependency of the absorption peak of retinochrome.

It has been suggested that the λ_{max} of rhodopsin could be explained on the basis of a protonated Schiff base of retinal and amino group of opsin, for the λ_{max} of a protonated Schiff base tends to shift to the longer wavelengths, when the protonated portion $-\text{C}=\text{N}-$, is at a longer distance from a

negative counter anion, so that the net charge on the protonated Schiff base is more positive^{4,5}. We believe that the absorption peak of retinochrome is also due to a protonated Schiff base of retinal with the protein like that of rhodopsin. The following mechanism can therefore be considered for the dependence of λ_{max} of retinochrome on salt concentration. First, there is a direct action of the salt on the primary binding site, Schiff base linkage. Anions such as chloride will concentrate at the protonated portion of the Schiff base linkage and decrease its net positive charge. Although the chromophore binding site in retinochrome is probably more exposed than in rhodopsin, the shift in λ_{max} of retinochrome is not adequately explained by such a direct action of anions, because it depends on the species of cation, as described.

A second explanation involves the indirect actions of salts on retinochrome. It is well known that salts such as LiCl and CaCl_2 at concentrations above 1 M cause the denaturation of some proteins⁶. Furthermore, in the bleaching of cattle rhodopsin, an increase in ionic strength facilitates the conformational change in the protein moiety that corresponds to the conversion from Meta I to Meta II⁷. Salt may affect some of the dissociated amino acids residues in the protein parts resulting in conforma-

tional change in protein parts. Such a mechanism is also probable in the case of retinochrome. If the increase in salt concentration causes conformation changes of the protein moiety and thereby decreases the distance between the protonated Schiff base and its counter anions this could result in the blue shift of λ_{max} of retinochrome to shorter wavelengths. On the other hand, λ_{max} of cephalopod rhodopsin, metarhodopsin and other visual pigments are not affected by salt concentration. This suggests that the chromophore binding site in retinochrome is not so deeply embedded in the protein moiety as are those of the visual pigments and/or that the protein moiety surrounding the binding site is very different in retinochrome and in the visual pigments.

Further investigations on the binding site of the chromophore of retinochrome in comparison with that of rhodopsin and metarhodopsin may help to clarify the interrelationships between retinochrome and rhodopsin.

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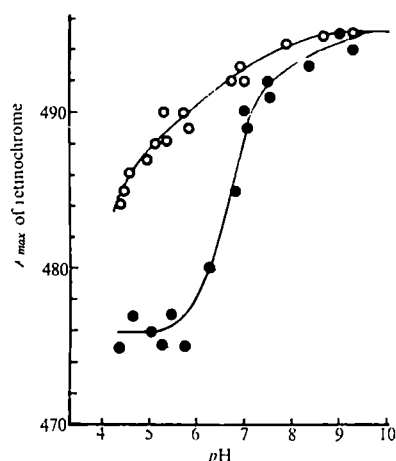
Non-constant evolution rates in amino acid sequences of guinea pig, chinchilla and coypu pancreatic ribonucleases

THE primary structures of the pancreatic ribonucleases from cow¹, rat², pig³, horse⁴, red deer and roe deer⁵, sheep⁶, goat⁶ and giraffe⁶ have been determined. From an analysis of these data we concluded that mammalian pancreatic ribonucleases have evolved with a rather constant evolution rate of about 1% substitution every 3 Myr (ref. 6). The validity of this conclusion, however, and possible exceptions have to be tested on a larger number of primary structures of mammalian ribonucleases.

As the amino acid sequences of the insulins from guinea pig and coypu differ markedly from those of other mammalian species^{10,11}, the amino acid sequences of other proteins from hystricomorph rodent species are of great interest. Here we describe the amino acid sequences of the pancreatic ribonucleases from the hystricomorph rodent species guinea pig (*Cavia porcellus*), chinchilla (*Chinchilla brevicaudata*) and coypu (*Myocastor coypus*). Bartos and Uziel¹² described the isolation of two ribonuclease components from guinea pig pancreas. We have reproduced this finding and demonstrated that both components differ in amino acid sequence.

Ribonucleases were isolated by extraction of pancreatic

Fig. 3 Dependence of λ_{max} of retinochrome on pH in digitonin solutions containing 0.01 M (○) and 1 M (●) NaCl . When the samples became turbid by the acidification or alkalisation, they were centrifuged 1 h at 28,000g at 4°C before measuring their absorption spectra.



tissue with 0.125 M sulphuric acid, precipitation with acetone, ammonium sulphate fractionation, gel filtration on Sephadex G-25 and repeated chromatography on CM-cellulose (according to Åqvist and Anfinsen¹³ and with a sodium acetate gradient⁵, respectively). The amino acid sequences have been determined on tryptic digests of the aminoethylated proteins. The tryptic peptides have been positioned in the sequence by homology with other pancreatic ribonucleases and with each other. Full details of the isolation of the enzymes, several properties and the sequence determinations will be described elsewhere.

The coypu pancreas has one carbohydrate-containing ribonuclease component. From guinea pig pancreas two ribonucleases have been obtained, which not only differ in the presence (RNase B) or absence of carbohydrate (RNase A), but also in amino acid sequence. From chinchilla pancreas two carbohydrate-containing ribonuclease components have been obtained—one homogeneous and the other heterogeneous. The latter differs from the first in being more acidic and exhibits heterogeneity both in its carbohydrate moiety (glycopeptides both with and without sialic acid have been isolated) and in amino acid sequence.

Table 1 Difference matrix of pancreatic ribonucleases

	Cow	Pig	Horse	Coypu	Chinchilla	Guinea pig A	Guinea pig B	Rat
Cow	—	20%	27%	27%	24%	25%	27%	34%
Pig	26	—	23%	25%	22%	27%	21%	35%
Horse	35	30	—	25%	21%	26%	28%	34%
Coypu	36	33	33	—	15%	24%	20%	40%
Chinchilla	32	29	28	19	—	21%	22%	36%
Guinea pig A	33	36	34	31	27	—	24%	39%
Guinea pig B	35	28	37	26½	28½	31	—	40%
Rat	44	46	45	52	47	51	52	—

Both the number and percentage differences are calculated from a hypothetical maximum chain length of 131 residues.

(probably glycine at position 32 has been partially substituted by aspartic acid).

The amino acid sequences of coypu, chinchilla and guinea pig ribonucleases A and B are shown in Fig. 1. Chinchilla and guinea pig ribonuclease A have a 'normal' chain length of 124 residues. Coypu and guinea pig ribonuclease B have additional amino acid residues at the C-terminus as has been found earlier in horse ribonuclease⁵.

The origin of both guinea pig ribonucleases must reside



Fig. 1 Amino acid sequences of the ribonucleases of coypu, chinchilla and guinea pig. Identical residues in the four sequences are enclosed in blocks. Asn*, asparagine with carbohydrate.

Position	:	1	9	16	21	23	32	64	76	78	86	89	92	102	103	113
ANCESTOR HYSTRICOMORPH RODENTS	:	LYS	GLU	GLY	THR	ALA	ARG	PRO	ASN	SER	LEU	SER	TYR	ALA	GLU	ASN
ANCESTOR COYPU AND CHINCHILLA	:	LYS	GLU	ARG	THR	ALA	SER	PRO	ASN	ASN	LEU	ASN	TYR	GLU	GLU	ASN
Coypu	:	Ser	Glu	Arg	Thr	Pro	Ser	Leu	Asn	Asn	Val	Asn	Tyr	Glu	Glu	Asn
Chinchilla	:	Lys	Gln	Ser	Thr	Ala	gly asp	Pro	Asn	Asn	Leu	Asn	Tyr	Glu	Asn	Asn
ANCESTOR GUINEA PIG	:	ALA	GLU	GLY	SER	ALA	ARG	PRO	TYR	SER	LEU	SER	PHE	ALA	GLN	ASN
Guinea pig A	:	Ala	Glu	Gly	Ser	Ala	Lys	Ser	Tyr	Ser	Leu	Gly	Phe	Ala	Gln	Asp
Guinea pig B	:	Ala	Gln	Glu	Asn	Ser	Arg	Pro leu	Tyr	Arg	Val	Ser	Phe	Ala	Gln	Lys

Fig 2 Amino acid residues in the ribonucleases of coypu, chinchilla, guinea pig (A and B) and the hypothetical ancestors of chinchilla and coypu ribonuclease, of the two guinea pig ribonucleases and of the hystricomorph rodent ribonucleases, at the 15 positions where three or four different residues are found in the four ribonucleases occurring now. The ancestral residues have been derived from the sequence data in Fig 1 and refs 1-9, and from the genetic code. Identical residues in coypu and chinchilla ribonuclease and in the two guinea pig ribonucleases are enclosed in blocks.

in a gene duplication. The many differences in amino acid sequence and also in other features make it unlikely that the two components represent alleles. The gene duplication may have occurred sometime before or after the divergence of the guinea pig from the other two hystricomorph rodent species investigated. In the first case we must assume that one of the two genes is a silent gene and does not come to expression in the other species or was lost again. In the other case the presence of two ribonuclease genes has been a novel acquisition in the line leading to the guinea pig, giving rise to new structural and functional possibilities which differ for the two gene products, resulting in an increased rate of acceptance of mutations. Here we provide evidence that the second possibility is more probable.

Table 1 is a difference matrix for the amino acid sequences of the ribonucleases from the hystricomorph rodents, cow, pig, horse and rat. Ribonucleases of species from different mammalian orders differ in about 30-35 positions (about 25%), with the exception of rat ribonuclease which differs in about 45-50 positions (about 35%) from all the others, including the hystricomorph rodent ribonucleases. Preliminary sequence information from the ribonuclease of the muskrat (a myomorph rodent species like the rat) indicates that the evolution rate of rat pancreatic ribonuclease has increased considerably after divergence of the cricetidae (including the muskrat) from the muridae (including the rat) (H van Dijk, A van den B, W Gaastra and J J B, unpublished).

The data in Table 1 show that the four hystricomorph rodent ribonucleases form a group of related sequences. But as a group they do not show the deviating behaviour from the homologous proteins of other mammalian species as has been encountered before with the insulins of the same species^{10,11}.

It is difficult to reconstruct the evolutionary history of amino acid sequences from a difference matrix if the evolution rates have not been constant and equal in all lines¹⁴. Therefore, we prefer to use an approximation of the ancestral sequence method^{15,16} for this purpose.

Excluding the additional residues at the C-terminus in coypu and guinea pig B, the 124 amino acid positions can be divided into three groups: (1) 85 out of 124 (69%) positions are identical in the four sequences (these are the blocked sequences in Fig 1) and are also, an indication of the relatively close relationship between them (Table 1); (2) 24 positions differ in only one of the four sequences, the other three are identical in these positions. The distribution of these 24 deviating residues between the four

sequences is as follows (Fig 1): three in coypu ribonuclease, four in chinchilla ribonuclease, ten in guinea pig ribonuclease A, seven in guinea pig ribonuclease B. These data point to an increased evolution rate in the two guinea pig ribonucleases as compared to the other two, but cannot provide us with the information necessary to deduce the evolutionary tree of the four sequences. (3) This goal must be accomplished with the data for the remaining 15 positions occupied by three or four different residues in the four sequences. The residues at these 15 positions are given separately in Fig 2. There are strikingly close relationships between the ribonucleases from chinchilla and coypu (seven identities) and the two guinea pig ribonucleases (five identities). Chinchilla and guinea pig ribonuclease A correspond in two positions, other combinations correspond in a smaller number of positions.

These data are the basis of the evolutionary history of the four hystricomorph rodent ribonucleases shown in

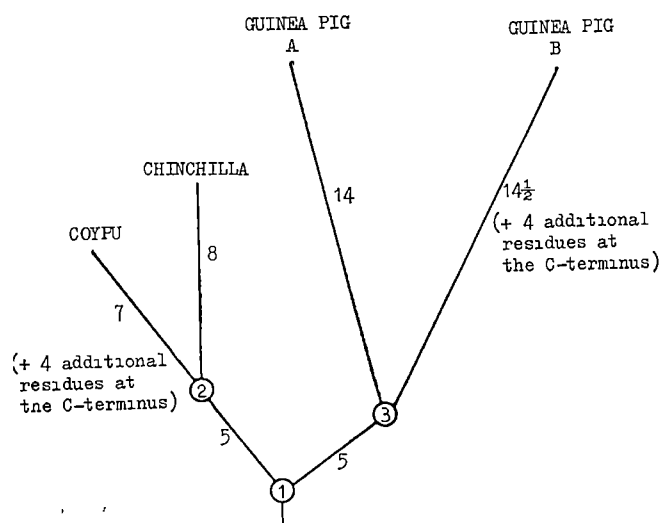


Fig 3 Evolutionary tree of the hystricomorph rodent ribonucleases. Node 1, hypothetical ancestor of the hystricomorph rodent ribonucleases; node 2, hypothetical ancestor of the ribonucleases of coypu and chinchilla; node 3, hypothetical ancestor of ribonuclease A and B of guinea pig. The number of amino acid substitutions between two sequences are given next to the lines connecting those sequences. The lengths of these lines are drawn in proportion to these numbers. It has been assumed for simplicity that the 24 positions which differ in only one of the four sequences (group 3 see text) are occupied in the three ancestral sequences by the residues which occur in three of the four sequences.

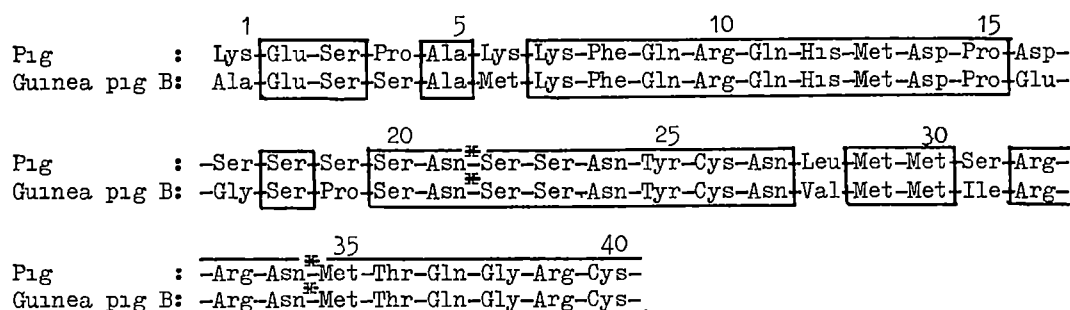


Fig. 4 Comparison of the N-terminal sequences of porcine and guinea pig ribonuclease B. Compare the similarities between these two sequences with the homologies in this part of the molecule of the hystricomorph rodent ribonucleases (Fig. 1) and between porcine and bovine ribonuclease³ Asn*, asparagine with carbohydrate

Fig. 3 Coypu and chinchilla ribonuclease have evolved with a rather normal evolution rate. The ancestral guinea pig ribonuclease has branched off considerably earlier than the divergence between coypu and chinchilla ribonuclease. The next event which took place was a gene duplication resulting in two guinea pig ribonucleases, which have diverged from each other with a considerably increased evolution rate.

The amino acid sequence of the carbohydrate attachment site Asn-21 is identical in porcine and guinea pig ribonuclease B. It is evident from the difference matrix (Table 1) that there are more similarities in structure between these two proteins (both sequences differ at 24 positions, excluding the four additional residues at the C-terminus in guinea pig B), so they differ significantly less than other ribonucleases from different mammalian orders. These structural similarities are predominantly found in the N-terminal part of the sequence (Fig. 4), where, as well as many identities, two strikingly conservative substitutions are also found at position 16 (Glu/Asp) and 28 (Val/Leu). Perhaps the formation of identical carbohydrate attachment sites at Asn-21 has necessitated the development of more structural similarities than the Asn-Ser-Ser (21-23) sequence. A point in case may be the formation of a hydrophobic surface on the protein near the attachment site of the carbohydrate. Two remarkable substitutions in guinea pig B are the valine at position 28 and the isoleucine at position 31 instead of the charged residues glutamic acid and lysine, respectively, which occur at these positions in the ribonuclease of the other hystricomorph rodents. These two residues are at neighbouring sites at the surface of the molecule (as can be deduced from a model of bovine ribonuclease S) and it is very likely that they are in contact with the carbohydrate chain attached to Asn-21. In porcine ribonuclease the positions 28 and 31 are occupied by the non-charged residues leucine (hydrophobic like Val-28 in guinea pig B) and serine, respectively. Thus, the structural similarity between porcine and guinea pig ribonuclease B may be a case of structural convergence of two homologous proteins which have developed identical secondary characteristics.

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A *cis*-dominant regulatory mutation affecting enzyme induction in the eukaryote *Aspergillus nidulans*

STUDIES ON *cis*-acting regulatory mutants (for example, operator and promoter mutants) in bacteria have been of crucial importance in understanding gene regulation¹⁻⁴. Similar mutants in eukaryote systems have not been extensively studied although there have been two reports of probable operator constitutive mutants in yeast^{5,6}. An understanding of eukaryote genetic organisation and gene regulation requires that mutants affecting the regulation of adjacent genes of defined function be isolated and characterised. This communication describes a mutant of *Aspergillus nidulans* that is altered in a site closely linked to the structural gene (*amdS*) for acetamidase (EC 3.5.1.4)^{7,8} and results in increased inducibility of this enzyme by acetamide. It is further shown that this mutation is *cis*-dominant in its effects.

In *Aspergillus nidulans*, lesions in the gene *areA* can result in inability to utilise a wide variety of nitrogen sources, including acetamide⁹. This gene is probably involved in regulation of enzyme synthesis by ammonium (ref 9 and M J H, unpublished). Strains containing the mutation, *areA217*, grow very weakly on acetamide (and many other compounds) as the sole nitrogen source in the presence of glucose or acetate as sole carbon sources, but grow normally on acetamide as

the sole carbon source. The original mutant was isolated from a strain containing the *areA217* lesion as a N-methyl-N'-nitro-nitrosoguanidine-induced revertant which could grow strongly on medium containing acetate as the sole carbon source with acetamide as the sole nitrogen source.

Genetic analysis has shown that the mutant contains a lesion, provisionally designated *amdI9*, which is closely linked to *amdS*, the structural gene for acetamidase, and that it retains the *areA217* mutation. An *areA*⁺, *amdI9* derivative obtained by crossing to a wild type strain, has been used extensively in further analyses.

Table 1 Growth properties of *amdI* strains on acetamide and acrylamide

Relevant genotype	Acetamide*	Acrylamide*	Acetamide†
<i>areA217, amdI</i> ⁺	—	—	+
<i>areA217, amdI9</i>	+	—	++
<i>areA</i> ⁺ , <i>amdI</i> ⁺	+	±	+
<i>areA</i> ⁺ , <i>amdI9</i>	++	±	++

Growth tests on solid medium at 37° C were carried out as described previously¹⁰. Growth symbols: ++, very strong growth, +, strong growth, ±, weak growth, —, background growth. Symbols on different media are not equivalent.

*Present as sole nitrogen sources (10 mM) in glucose-minimal medium.

†Present as sole carbon and nitrogen source (50 mM).

The *amdI9* lesion results in increased growth on acetamide, either as a carbon or as a nitrogen source (Table 1), but does not affect growth on other nitrogen sources. Acrylamide is a non-inducing substrate for acetamidase¹¹. Therefore the finding that strains containing *amdI9* do not grow better than *amdI*⁺ strains on acrylamide as the sole nitrogen source in glucose medium, indicated that inducibility of the acetamidase might be affected by *amdI9*. The enzyme results shown in Table 2 support this proposal. Strains containing *amdI9* have higher acetamidase activities than *amdI*⁺ strains under conditions of induction by acetamide but similar activities to *amdI*⁺ strains under non-induced conditions. Therefore *amdI9* appears to result in increased acetamidase induction by acetamide.

Table 2 Acetamidase activities of *amdI* strains

Final growth conditions*		Relevant genotype			
Glucose	Acetamide	<i>areA217, amdI</i> ⁺	<i>areA217, amdI9</i>	<i>areA</i> ⁺ , <i>amdI</i> ⁺	<i>areA</i> ⁺ , <i>amdI9</i>
Present	Absent	0	0	14	13
Present	Present	0	22	21	34
Absent	Absent	35	43	35	39
Absent	Present	81	270	60	274

Mycelia for enzyme assays grown, extracted and assayed by methods described previously^{12,13}. Units of activity = nmol ammonium per min per mg protein.

*Mycelia grown for 16 h in glucose-10 mM ammonium medium and then transferred to these media for a further 4 h before harvesting.

To see if the effects of *amdI9* are limited to the *amdS* gene in the *cis* position it was necessary to obtain strains of genotype *amdI9, amdS*[−] (where *amdS*[−] represents a structural gene lesion) by isolating spontaneous mutants of *amdI9* strains which are resistant to fluoroacetamide. This technique readily yields *amdS*[−] mutants with low acetamidase activities⁷. In this way a number of *amdI9, amdS*[−] strains have been isolated. The presence of lesions in *amdS* was confirmed by complementation tests in heterokaryons and diploids with known *amdS*[−] mutants, as well as the finding of close linkage to known *amdS*[−] mutants. In the cases of the *amdI9, amdS*[−] mutants reported here, the retention of the *amdI9* lesion has been shown by the recovery of *amdI9, amdS*⁺ recombinants in crosses. Heterozygous diploids with *amdI9* in *cis* or in *trans* to *amdS*⁺ genes have been constructed and tested for acetamide utilisation and acetamidase activities (Table 3). The *amdI9* lesion is *cis*-dominant to *amdI*⁺ in such diploids, with strong growth on acetamide and elevated acetamidase activities occurring only when *amdI9* is *cis* to *amdS*⁺.

amdI9 strains produce an acetamidase that is indistinguishable from the enzyme of *amdI*⁺ strains in electrophoretic

Table 3 *Cis* dominance of *amdI9* in heterozygous diploids

Relevant <i>amd</i> genotype	Growth on acetamide*	Acetamidase activity†
<i>I</i> ⁺ <i>S</i> ⁺	+	60
<i>I9S</i> ⁺	++	274
<i>I9S</i> [−] <i>91</i>	—	3
<i>I9S</i> [−] <i>106</i>	—	4
<i>I</i> ⁺ <i>S</i> [−] <i>11</i>	—	3
<i>I9S</i> ⁺ / <i>I</i> ⁺ <i>S</i> ⁺	++	208
<i>I9S</i> ⁺ / <i>I</i> ⁺ <i>S</i> [−] <i>11</i>	++	200
<i>I9S</i> [−] <i>91</i> / <i>I</i> ⁺ <i>S</i> ⁺	+	37
<i>I9S</i> [−] <i>106</i> / <i>I</i> ⁺ <i>S</i> ⁺	+	30
<i>I9S</i> [−] <i>91</i> / <i>I</i> ⁺ <i>S</i> [−] <i>11</i>	—	0

*Acetamide (10 mM) in solid glucose-minimal medium. Symbols as for Table 1.

†Mycelia grown for 16 h in glucose-10 mM ammonium medium and then transferred to minimal medium containing 20 mM acetamide as the sole carbon and nitrogen source for 4 h before harvesting. Units as for Table 2.

mobility in starch gels, thermolability and substrate specificity (M J H, unpublished). This, together with higher enzyme levels found only under induction conditions, makes it likely that *amdI9* affects acetamidase regulation rather than structure. The simplest hypothesis to account for this data is that *amdI9* affects a regulatory site necessary for interaction with a regulatory molecule involved in induction by acetamide. This site is altered by the *amdI9* mutation such that inducibility is greatly increased. Although a number of possible regulatory genes affecting the acetamidase have been found (refs 7–9, 11, 13 and M J H, unpublished), it is not yet clear which of these is involved in induction by acetamide. The hypothetical regulatory molecule could have a negative action (repressor) and in this case *amdI9* would cause repressor binding to be weaker. Alternatively (and perhaps more likely) the regulatory molecule could have a positive action (activator), with *amdI9* allowing a more efficient activation of enzyme synthesis. Studies on the effects of *amdI9* on the general control mechanisms, such as ammonium repression and catabolite repression^{8,12,13}, which affect acetamidase, are in progress. These,

together with genetic fine structure analysis, should provide important information regarding the relationship between the regulatory sites influencing the activity of the adjacent structural gene.

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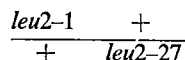
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Role of premeiotic replication in gene conversion

CURRENT molecular models of meiotic recombination have attempted to explain both classical reciprocal recombination (crossing over) and non-reciprocal intragenic recombination (gene conversion), with a single unified mechanism^{1,2}. An important assumption inherent in such models is that both types of recombination occur during the prophase following premeiotic chromosome replication. To date, however, only crossing over (chiasma) has been convincingly shown to occur during meiotic prophase³. Gene conversion, which has been most extensively studied in the ascomycete fungi⁴, has, for a number of cogent reasons⁵, also been assumed to occur during prophase, yet direct evidence supporting this critical point is lacking. In fact, in an important paper⁶, concerning recombination in yeast, evidence was presented indicating that intragenic recombination and premeiotic replication coincided, a finding which argues against much current speculation. To clarify this situation we simultaneously measured premeiotic replication and gene conversion in the yeast *Saccharomyces cerevisiae*, and examined the genetic consequences of inhibiting DNA synthesis with hydroxyurea (HU). We show that during the premeiotic S phase cells acquire a 'potential' for completing gene conversion, but that the actual formation of stable intragenic recombinants occurs after replication and depends on a period of postreplicative DNA synthesis.

In yeast, meiosis is induced by transferring logarithmically growing cells to nitrogen-free sporulation medium⁷. The kinetics of various meiotic events are shown for diploid strain 4579 (Fig. 1a). Since this strain is heteroallelic for a non-complementing pair of mutations at the *leucine-2* locus



gene conversion could be monitored by the appearance of prototrophic recombinants (+ +) on leucine-free selective medium. The *leu2-1* and 2-27 alleles were chosen because genetic analysis⁸ revealed that prototrophs arose by gene conversion rather than by reciprocal recombination. The recovery of prototrophs coincided rather closely with the period of premeiotic replication (6-15 h) (Fig. 1a). Data from seven experiments correlating the extent of DNA synthesis with the recovery of recombinants are given in Fig. 1b, clearly showing the close temporal relationship between the two events. Coincidence between replication and intragenic recombination was also seen in diploids heteroallelic for *arginine-4*, *leucine-1*, *uracil-3*, and *adenine-8*. Sporulation, which signals the completion of meiosis, extended within the population from 14-30 h.

We considered two alternative explanations for the temporal coincidence between replication and the recovery of gene convertants: (i) gene conversion occurs during premeiotic replication, or (ii) premeiotic replication coincides with 'commitment' of the cell to a developmental pathway which includes gene conversion, but the actual conversion event takes place later, during the postreplicative interval. The second alternative exists because of the indirect way in which recombination is measured; in practice, conversion is detected by counting prototrophic colonies which appear several days after meiotic cells are plated on selective medium. Thus the exact time of formation of a recombinant gene is obscured⁹, conceivably it could have been many hours after a 'committed' cell was first plated on selective medium.

To distinguish between the two alternatives we used hydroxyurea (HU), a specific reversible inhibitor of DNA synthesis¹⁰. We reasoned that if recombinants were actually generated

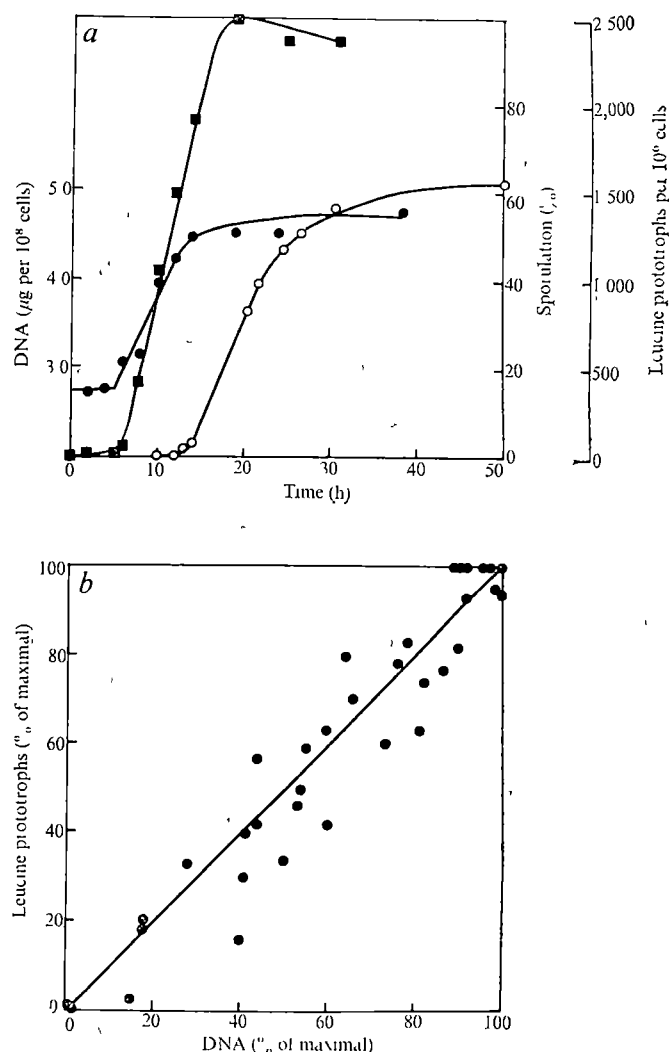


Fig. 1 Coincidence between recovery of gene convertants and replication during meiosis. Strain 4579 has the following genotype

<i>his4</i>	<i>leu2-1</i>	+	<i>α</i>	<i>thr4</i>	<i>ade2</i>	<i>met2</i>	<i>ura3</i>
+	+	<i>leu2-27</i>	<i>a</i>	+	<i>ade2</i>	<i>met2</i>	<i>ura3</i>

Media and procedures used for growth and induction of meiosis (sporulation) were previously described¹⁴; incubations were at 30°C. a, At zero time (*t* = 0) a growing culture was collected, washed, and suspended in acetate sporulation medium. At intervals, samples were removed to measure simultaneously (i) DNA synthesis per cell (●), (ii) the frequency of leucine prototrophic recombinants (■), and (iii) the percentage of asci (○). DNA was determined using a semimicro modification of the procedure of Croes¹⁵ (details to be published elsewhere). Leucine prototrophs were monitored by plating diluted culture samples on to leucine-free synthetic medium¹⁴. Ascus formation was determined by phase contrast microscopy. b, Data from seven independent experiments relating DNA synthesis and prototroph recovery were combined, as follows: for each experiment the total increase in DNA between *t* = 2 h and *t* = 36 h was set equal to 100%, similarly the maximal increase in prototrophs over this interval was also set to 100%. For each experimental point the percentage increase in DNA was plotted against the corresponding value for the increase in prototrophs.

during replication, then exposure to HU after replication was complete would not affect those recombinants already formed. In contrast, if gene conversion followed replication, then HU added to cells after replication might inhibit recombination by interfering with the localised, repair-type DNA synthesis which would be necessary for gene conversion¹¹.

In preliminary experiments we demonstrated that 100 mM HU inhibited both the initiation and continuation of DNA synthesis in meiotic cells. But, increases in mass, and protein

and RNA synthesis continued in essentially normal fashion (for up to 18 h) in cultures treated with HU. Thus, in meiotic cells as in mitotic cells¹⁰, HU selectivity inhibited DNA synthesis. The effects of HU on recombination are shown in Fig 2, addition of the drug before replication completely blocked the appearance of meiotic recombinants, however, the low frequency of leucine prototrophs pre-existing in the population was not affected. When HU was added any time during replication, recombination was quickly halted, in addition, with continued incubation in HU there followed a rapid and extensive decline in the number of recombinants initially present at the time of drug addition. Eventually, the rapid loss of recombinants stopped, and their frequency reached a stable value which depended on the time of drug addition. As shown in Fig 2 the decline in recombinants could not be attributed to a generalised toxic effect: viability even after prolonged drug exposure was > 95% HU, added during replication, caused similar declines in the number of recombinants in diploids heteroallelic for unrelated loci (for example *arg-4*, *leu-1*, and *ura-3*). In no case could the decline be attributed to losses in viability.

Was the dramatic HU-induced decline in recombinant frequency associated with inhibition of ongoing chromosome replication (the premeiotic S phase), or with inhibition of postreplicative DNA synthesis? To answer this we had to determine the time when premeiotic S phase was completed in the asynchronous population of developing cells. Completion of replication was evaluated by blocking DNA synthesis (with HU) at various times and examining the ability of inhibited cells to complete meiosis, as judged by the formation of mature asci (Fig 3). We considered sporulation a functional test for the completion of replication. Curve *a* of Fig 3 depicts the final percentage of asci obtained in HU-inhibited cultures plotted against the time of drug addition. Presumably, curve *a* represents the kinetics of completion of replication in the population. Comparing curve *a* with the effects of HU on

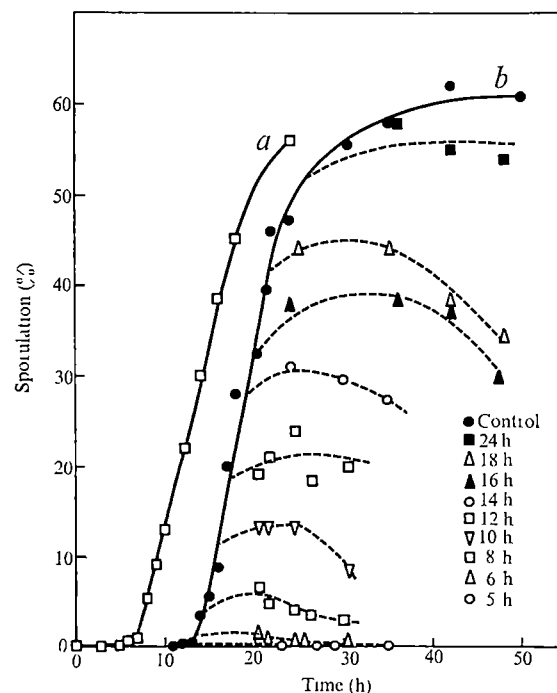


Fig 3 Effects of hydroxyurea on sporulation. A meiotic culture of 4579 was established as in Fig 1. Sporulation in this culture (● = control), is shown as curve *b* (solid line). At the times indicated on the figure (and at $t = 0, 3, 7$, and 9 h, detailed data not shown), portions of the control culture were transferred to separate flasks containing HU (final concentration = 100 mM). During subsequent incubation in HU, samples from each culture were removed, fixed in formaldehyde, and scored for percentage sporulation; these data are shown as a series of broken lines. Note that after prolonged incubation in HU the percentage of asci begins to decline. In curve *a* (□), the maximal level of sporulation reached in each of the HU-treated cultures is shown plotted against the time when the cells were first exposed to the drug.

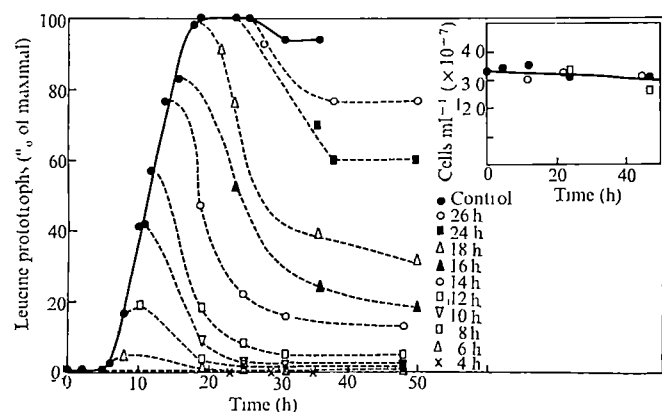


Fig 2 Effects of hydroxyurea on meiotic gene conversion and viability. A meiotic culture of 4579 was established as in Fig 1. Recovery of prototrophs in the control culture (●) is shown as a solid line. At the times indicated on the figure, portions of the control culture were transferred to separate flasks containing sufficient 2.5 M hydroxyurea to yield a final concentration of 100 mM. During subsequent incubation in HU, samples from each culture were plated to determine the frequency of remaining prototrophs; these values are shown as a series of broken lines. For simplicity, the maximal prototroph frequency in the control was set to 100%, and the absolute frequencies of prototrophs in the HU-treated cultures were converted to percentages of this value. Hydroxyurea (Squibb) was dissolved in H₂O, and filter sterilised just before use. Inset: effects of extended HU exposure on survival of meiotic cells. At $t = 5$ h and $t = 12$ h, portions of a meiotic culture (control) were transferred to flasks containing HU (final concentration = 100 mM). At intervals, samples from the control and HU-treated cultures were removed, sonicated briefly to disrupt clumps, diluted, and plated on complete medium¹⁴. Colonies were counted after 5 d. ●, Control, ○, HU at $t = 5$ h, □, HU at $t = 12$ h.

recombination (Fig 2), revealed that the fraction of cells (relative to the control frequency) which completed replication was far in excess of the fraction of cells which formed stable (HU-resistant) recombinants. For example, at $t = 12$ h ~ 1/3 of the cells had completed replication, yet addition of HU at this time revealed that of the apparent recombinants (55%) only 5% were stable. This suggests that gene conversion cannot be consummated during the premeiotic S phase, but only later. In addition, the sensitivity of recombination to HU, in cells which have completed replication, indicates that successful gene conversion requires some postreplicative DNA synthesis.

Our conclusion that gene conversion occurred after replication depended on at least two assumptions: (i) that HU acted quickly to stop replication, and therefore, gave an accurate estimate for the time of completion of replication, and (ii) that ascus formation in HU is a valid functional test for the presence of a fully replicated genome.

To confirm independently that HU could provide an accurate estimate of the completion of replication (Fig 3) in a population, we examined the consequences of blocking DNA synthesis using one of Hartwell's temperature-sensitive replication defective mutations¹². Strain Z-198 (Fig 4) is homozygous for a thermal lesion in gene *cdc8*, which controls a step required for DNA polymerisation. During meiosis, a shift from a permissive temperature (23°C) to a restrictive temperature (34.5°C) immediately blocked replication. A culture of Z-198 was grown and transferred to sporulation medium at 23°C, at intervals duplicate samples were either shifted to 34.5°C, or put into HU at 23°C (Fig 4). The final percentage sporulation attained plotted against the time of the HU or high-temperature challenge are compared in Fig 4. Both treatments yielded virtually identical curves, and thus, provided the same estimate for the time of completion of replication in the population.

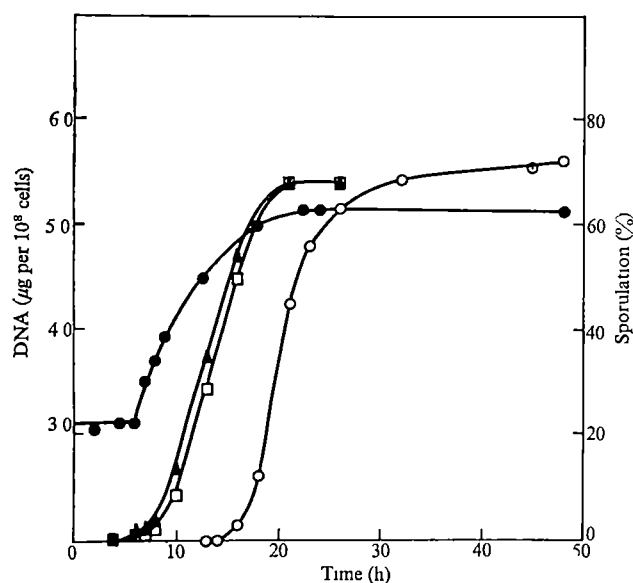


Fig. 4 Effects of hydroxyurea and restrictive temperature on sporulation of strain Z-198

Thermosensitive strain Z-198 has the following genotype

<i>cdc8-1</i>	<i>ura1</i>	<i>his4</i>	<i>leu2</i>	<i>a</i>	<i>thr4</i>	<i>ade2</i>	<i>tyr1</i>
<i>cdc8-1</i>	<i>ura1</i>	+	+	<i>a</i>	+	+	+
<i>his7</i>	<i>lys2</i>	+	+	<i>ade1</i>	+		
+	-	<i>met2</i>	<i>trp5</i>	<i>ade1</i>	<i>gal1</i>		

It was constructed by mating Hartwell's *cdc8-1* parent strain¹² to a thermoresistant haploid of opposite mating type (*x1069-2D*), and selecting for a thermosensitive mitotic segregant following a sublethal dose of ultraviolet irradiation¹⁶. Homozygosity for *cdc8-1* was confirmed by complementation tests¹³. A meiotic culture of Z-198 was established as in Fig. 1, except that growth and sporulation of the control culture were conducted at 23°C. Premeiotic DNA synthesis (●), and sporulation (○), were determined as in Fig. 1. At intervals during meiosis duplicate samples were removed from the control culture and transferred, either to a flask prewarmed and maintained at 34.5°C, or to a flask maintained at 23°C, but containing HU (100 mM). Sporulation was monitored for an extended period (48 h) in each of the HU and heat-treated cultures. The maximal percent sporulation reached in each culture is shown plotted against the time when the cultures were first exposed to the HU (▲) or the restrictive temperature (□).

Evidence that the asci formed in HU contained viable ascospores was obtained as follows: meiotic cells (of strain 4579) were exposed to HU, at $t = 12$ h, and allowed to complete sporulation in the presence of the drug. The resulting asci were dissected, and the individual spores tested for survival on complete medium¹³. Viability for the HU-treated asci (86%), was similar to that in mature asci from a control culture never exposed to the drug (90%). Thus asci formed in HU contained viable haploid cells, presumably originating by replication of the original diploid genome.

Our primary objective was to reconcile data which suggested that gene conversion might coincide with replication, and current molecular models which assume that conversion (as well as reciprocal recombination) takes place after replication, during meiotic prophase. We have shown that while 'potential' intragenic recombinants are recoverable during premeiotic replication, stable recombinants are only formed considerably after the premeiotic S phase. These results are entirely consistent with current models.

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Increased sensitivity of lymphocyte Δ^1 -pyrroline-5-carboxylate reductase to inhibition by proline with transformation

THE mitogen-induced transformation of resting lymphocytes to proliferating blast forms is associated with a marked increase in amino-acid uptake¹, protein synthesis² and enzyme activities³. There is little information, however, on the effect of transformation on enzymes of amino-acid metabolism. We therefore examined the biosynthetic and degradative enzymes of the non-essential amino acid, proline⁴, in resting and transformed lymphocytes. We found a selective increase of proline biosynthetic enzymes in association with transformation. It is interesting that transformation also produced qualitative changes in Δ^1 -pyrroline-5-carboxylate reductase, the enzyme which catalyses the committed step in proline synthesis.

Lymphocytes from normal human donors were prepared routinely by dextran sedimentation of heparinized whole blood or by separation of whole blood on Ficoll-Hypaque gradients. The collected leukocytes were then washed in balanced salt solution and cultured at a cell density of 0.5×10^6 mononuclear leukocytes per ml of RPMI 1640 medium supplemented with 10% autologous plasma, 4 mM glutamine, penicillin (50 U ml⁻¹) and streptomycin (50 μ g ml⁻¹). In each experiment half the cells were cultured with phytohemagglutinin (Burroughs Wellcome) at a concentration of 1 μ g ml⁻¹ (optimum concentration of this preparation as determined by thymidine incorporation) and are referred to as PHA cells, the remaining cells were cultured without PHA and are referred to as control cells. After specified periods of culture, cells were collected by centrifugation, washed three times in balanced salt solution at 4°C and disrupted by sonication in 1 ml of buffer appropriate for each enzyme assay. Enzyme activities of the lymphocyte sonicates were determined by radioisotopic assay methods as previously described⁵⁻⁸. Protein was determined by the method of Lowry⁹. In all instances enzyme specific activity is expressed as nmol product per h per mg protein.

PHA transformation produced marked changes in the enzymes of proline biosynthesis (Fig 1). Twelve hours after the addition of PHA the specific activity of the proline biosynthetic enzyme, Δ^1 -pyrroline-5-carboxylate reductase (PCA reductase), began to increase considerably. Seventy-two hours after the addition of PHA, the specific activities of the biosynthetic enzymes, ornithine- δ -transaminase (OTA) and PCA reductase, increased to levels at least fifteen times those in either cultured control cells or fresh peripheral lymphocytes. In contrast, the proline degradative enzymes did not follow this pattern. With 72 h of PHA-stimulated mitogenesis, the specific activity of PCA dehydrogenase remained unchanged while proline oxidase activity remained undetectable in either sonicates or mitochondrial preparations. For proline oxidase we used a radioisotopic method able to detect less than 5 pmol of product. Thus, only the biosynthetic enzymes of proline metabolism are increased with lymphocyte activation.

The marked increase in PCA reductase was of special interest to us. We showed previously that PCA reductase exists in two forms: one sensitive and one insensitive to feedback inhibition by proline¹⁰. Enzyme obtained from resting cells in animal tissues (rat liver, brain, kidney) is relatively insensitive to proline inhibition with less than 50% inhibition at 10^{-2} M proline. In contrast, PCA reductase from cells growing in culture (human fibroblasts, Chinese hamster ovary and lung cells and rat liver cells) is always markedly sensitive to proline inhibition with 50% inhibition at 2×10^{-4} M proline. Thus we considered whether the increase in PCA reductase in the transformed lymphocyte would be accompanied by a change in the proline sensitivity of the enzyme. Figure 2 shows the proline inhibition

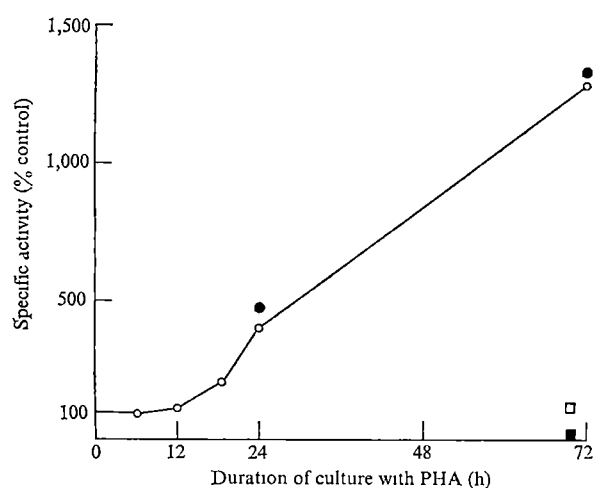


Fig 1 Effect of phytohaemagglutinin (PHA) transformation on the enzymes of proline biosynthesis and degradation. Data are expressed as percentage change over control cells incubated without PHA. Each point represents the mean of at least three determinations performed on extracts from three separate cultures. PCA reductase (○), ornithine transaminase (●), PCA dehydrogenase (□), proline oxidase (■).

of PCA reductase from control and PHA cells after culture for 72 h. The control cell enzyme was relatively insensitive to proline being inhibited only about 30% by 10^{-3} M proline. The same curve of proline inhibition was obtained when either fresh peripheral lymphocytes or cells purified to greater than 99% lymphocytes were the enzyme source. In contrast, the PHA cell enzyme was markedly more proline-sensitive, with 50% inhibition by 2×10^{-4} M proline and 85% inhibition by 10^{-3} M proline. Clearly in addition to a 15-fold increase in specific activity with lymphocyte transformation, PCA reductase became markedly more sensitive to inhibition by proline. The slight inhibition of the control enzyme by proline may have resulted from the presence of a small amount of the proline-sensitive form of the enzyme in resting lymphocytes or from the presence of 1–2% activated lymphocytes in the control cell population.

The marked difference in proline sensitivity of PCA reductase

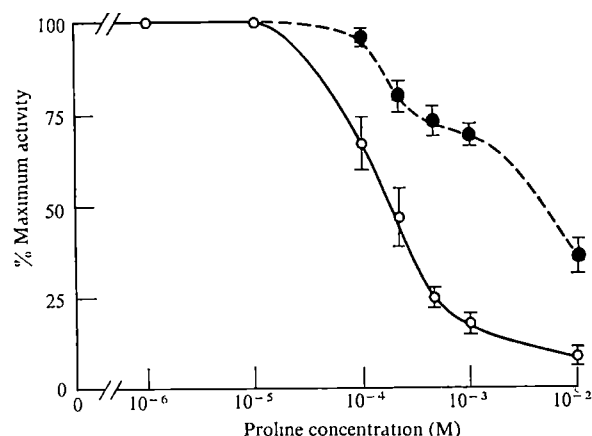


Fig. 2 Proline inhibition of PCA reductase. PCA reductase activities in extracts of 72-h PHA cells (○) and 72-h control cells (●) were assayed with various concentrations of proline. The assay mixture included Δ^1 -pyrroline-5-carboxylate, 0.045 mM (in the L configuration), NADH, 0.68 mM, phosphate buffer 0.1 M, pH 6.8, and 20 μ g of extract protein. The mixture was incubated at 37°C for 10 min. Each point represents the mean \pm s.e.m. of three determinations performed on extracts from three cultures.

in 72-h transformed lymphocytes may represent the selective synthesis of proline-sensitive PCA reductase. In support of this hypothesis, we found that the time course of change in sensitivity to inhibition by proline paralleled the increase in PCA reductase activity, both showing a 12-h lag after addition of PHA. Furthermore, the total increase in PCA reductase specific activity with transformation represents an increase only in the proline-sensitive activity. The proline-insensitive activity (activity in the presence of 1×10^{-3} M proline) did not change significantly during 72 h of PHA transformation.

If the observed 12-h lag was related to the synthesis of mRNA coding for the proline-sensitive reductase and subsequent accumulation of the new enzyme, we should have been able to block the appearance of the enzyme with inhibitors of RNA and protein synthesis. When either actinomycin D (5 μ g ml⁻¹) or cycloheximide (0.05 mM) was added to PHA cells at time zero and cells were collected 18 h later, both the increase in PCA reductase activity and the appearance of proline sensitivity were blocked (Fig 3). The PCA reductase activity of control cells was unaltered by a comparable exposure to inhibitors. Furthermore, actinomycin D added 8 h after addition of PHA in an otherwise similar experiment (data not shown) did not prevent increases in either PCA reductase activity or sensitivity to inhibition by proline. Therefore, these alterations in PCA reductase activity require both RNA and protein synthesis.

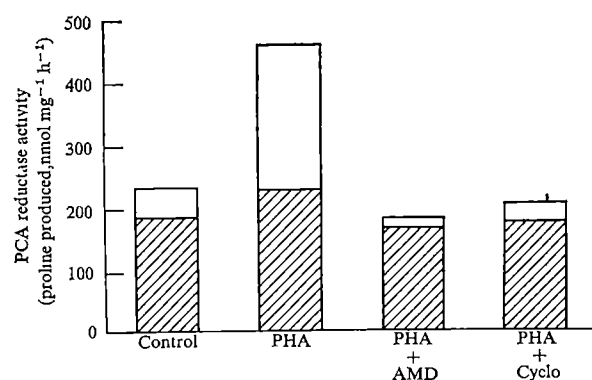


Fig. 3 The effect of actinomycin D and cycloheximide on PHA-induced increases in PCA reductase. Lymphocytes were cultured for 18 h with PHA, PHA plus actinomycin D, (5 μ g ml⁻¹) or PHA plus cycloheximide (0.05 mM). The bars represent total enzyme activity whereas the cross-hatched areas represent activities in the presence of 10^{-3} M proline. Data represent the mean of at least three determinations performed on extracts from three cultures.

The most straightforward explanation of these data would involve the preferential synthesis of a proline-inhibitable isozyme of PCA reductase in association with the change from resting to actively growing lymphocytes. This hypothesis is consistent with our work demonstrating the occurrence of proline-insensitive PCA reductase in resting cells obtained from animal tissues and proline-inhibitable reductase obtained from cells adapted for rapid growth in tissue culture¹⁰. Confirmation of this hypothesis will require direct demonstration of two forms of PCA reductase.

The accumulation of a proline-sensitive PCA reductase is beneficial to the activated lymphocyte in two ways. The proline synthetic capability is increased to meet the demands of increased protein synthesis and the proline sensitivity of this enzyme makes proline synthesis subject to negative feedback control. Thus, in the activated lymphocyte if exogenous proline were adequate to meet the demands of increased protein synthesis, PCA reductase would be inhibited, sparing proline precursors for use elsewhere in the cell.

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Human immunoglobulins with a1 allotypic determinants of rabbit immunoglobulin heavy chains

ALLOTYPIC genetic variations in the variable (V) part of immunoglobulin (Ig) chains have been clearly identified only in the domestic rabbit. Three allotypic specificities, a1, a2 and a3, controlled by allelic genes, *a*¹, *a*² and *a*³, at the *a* locus, are present on the variable region of heavy chains of all classes (IgM, IgG, IgA and IgE)¹⁻³. These allotypic specificities reflect multiple amino acid differences in the V region of Ig heavy chains⁴.

Subgroups also differ in the amino acid sequence in the V region. The heavy chains bearing the specificities controlled by the *a* locus represent a heavy chain subgroup designated V_Ha which accounts for 70-90% of the Ig heavy chains in the rabbit. The N-terminal amino acid sequence of pooled Ig heavy chains of the V_HIII subgroup from each of nine different species (including cat, dog, human, mouse, rat, guinea pig, mink, seal and sea lion), revealed striking similarities⁵. With one exception (cat), the sequence in any species did not differ from the sequence in any other species by more than four of the N-terminal 24 amino-acid residues⁵. Also, Rodkey and

Hansen⁶ found that some of the a2 and a3 allotypic specificities are present on the Fab fragment of IgG from cottontails and jack rabbits which are species closely related to the domestic rabbit. These observations led us to investigate whether anti-allotypic antisera, directed against the allotypic specificities present on the V part of rabbit heavy chains would react with human immunoglobulins. This report deals with the reaction of anti-a1 anti-allotype antiserum with human IgG (Hu IgG).

Pooled Hu IgG (FR II, Pentex, Kankakee, Illinois) was labelled with ¹²⁵I (ref. 7) and tested for the presence of the a1, a2 and a3 determinants by an indirect precipitation assay. Rabbit anti-a1, anti-a2 or anti-a3 antiserum was added to 0.1 µg ¹²⁵I-IgG, after 30 min at 37° C, goat anti-rabbit IgG antiserum (previously adsorbed with a Sepharose immunosorbent containing Hu IgG) was added to precipitate the complexes formed by the reaction of the anti-allotype antiserum and the ¹²⁵I-Hu IgG. After 24 h, the resultant precipitate

Table 1 Percentage precipitation of ¹²⁵I-labelled rabbit IgG or Fab_γ by rabbit anti-a1 antibody eluted from a Sepharose immunosorbent containing human IgG

Rabbit	Allotypes	¹²⁵ I-antigen	Radioactivity precipitated (%)
H209-3	a1,b5	IgG	85
		Fab _γ *	67
2L280-3	a1,b5	Fab _γ	75
F280-3	a1,b5	IgG	81
		Fab _γ	62
L244-1	a1,b5	IgG	89
		Fab _γ	68
F83	a2,b5	IgG	4
L16-1	a2,b5	IgG	5
03485	a3,b5	IgG	3

*The Fab_γ fragments were prepared by papain digestion of IgG followed by CM-cellulose chromatography as described by Porter¹³, the Fab I fragments were used as the antigens.

was washed, the radioactivity in the supernatant fluids and in the precipitates was determined and the percentage radioactivity precipitated was calculated⁸. Anti-a1 precipitated 71% of the radioactivity of the Hu IgG whereas anti-a2 and anti-a3 precipitated less than 5% of the radioactivity.

Those anti-a1 antibody molecules which reacted with Hu IgG were isolated from a Sepharose immunosorbent column containing pooled Hu IgG⁹. The anti-a1 antibody, reacting with Hu IgG, was used for the analysis of Hu IgG samples.

Hu IgG was isolated from serum samples of nine individuals by a combination of salt precipitation with Na₂SO₄ and DEAE-cellulose chromatography^{10,11}. The purified IgG samples were labelled with ¹²⁵I (ref. 7) and were shown to be more than 95% Ig by quantitative precipitation with rabbit anti-Hu IgG. These labelled IgG samples were reacted with the specifically purified anti-a1 by the quantitative radioprecipitation assay described above, from 45 to 70% (average, 61%) of the radioactivity was precipitated. In controls where normal (unimmunised) rabbit serum was substituted, for anti-a1 less than 9% of the radioactivity was precipitated.

To determine if the a1 specificity present on Hu IgG was a genetic variant or was present in all individuals, plasma samples from 270 individuals were tested for the presence of a1 specificities by a quantitative inhibition of precipitation assay¹². The 270 plasma samples were obtained from the clinical laboratories of our University Hospital and 5γ of a plasma sample was added to a tube containing 0.05 µg ¹²⁵I-Hu IgG (from an individual). An amount of anti-a1 that was slightly less than that needed to precipitate all of the ¹²⁵I-Hu IgG was added and the tubes were incubated for 30 min at 37° C, then, goat anti-rabbit IgG was added to precipitate the anti-a1 and any Hu IgG bound to the anti-a1. The percentage radioactivity precipitated was determined and the percentage in-

Table 2 Percent precipitation of ^{125}I -labelled human Fab_γ and F(ab')_2 fragments by anti-a1 anti-allotype antibody, anti-Hu IgG antiserum and anti-Hu Fc_γ antiserum*

Person	Labelled fragment	Precipitation by antibody to		
		a1 %	Hu IgG %	Hu Fc_γ %
WJH	F(ab')_2	36	79	1
UNK	Fab_γ	40	90	3

*Anti-Hu IgG has antibodies directed toward light chains and heavy chains. Anti-Hu IgG was made specific for determinants on the Fc part of IgG by passing the antiserum through a Sepharose immunosorbent column containing F(ab')_2 fragments of normal pooled human IgG

hibition was then calculated for each plasma sample. All 270 plasma samples completely inhibited the reaction of anti-a1 with Hu IgG, thus, all of the human sera tested possessed the a1 specificity.

That the specifically purified anti-a1 still reacted with the a1 determinants of the rabbit was shown by its ability to react with most (81–89%) of the IgG molecules as well as with the Fab_γ fragment obtained from rabbits with the genotype $a^1a^1b^5b^5$ (b^4 and b^5 are allelic genes at the b locus, these genes control the synthesis of rabbit b4 and b5 light chains, respectively). The purified anti-a1 did not precipitate the IgG molecules obtained from two $a^2a^2b^5b^5$ rabbits nor from one $a^3a^3b^5b^5$ rabbit (Table 1). These assays were done by the quantitative precipitation analysis as described above except that anti-b4, rather than goat anti-rabbit IgG, was used as the indirect reagent (the anti-a1 had been prepared in a b^4b^4 rabbit thus permitting the use of anti-b4 as an indirect reagent).

Human Fab_γ fragment was prepared by papain digestion¹⁴ of Hu IgG obtained from one individual (UNK) and F(ab')_2 fragment was prepared by pepsin digestion¹⁵ of Hu IgG obtained from another individual (WJH). Analysis of these fragments by the radioprecipitation assay revealed that the a1 determinant is present on the Fab_γ (40%) and the F(ab')_2 (36%) fragments (Table 2) as well as on the intact IgG (47%) of WJH. That the F(ab')_2 and the Fab_γ fragments were not contaminated with Fc fragments is shown by the observation that anti- Fc_γ did not react with the F(ab')_2 nor with the Fab_γ fragments, that is, less than 5% of the radioactivity was precipitated (Table 2).

Not all anti-a1 antisera reacted with Hu IgG. Of the anti-a1 antisera obtained from 5 different rabbits (E208-3, K158-3, 53923, H207-2, and J314-1) only E208-3 and K158-3 reacted with Hu IgG. The explanation for this observation is currently not clear. As not all anti-a1 antisera reacted with Hu IgG, we have recently examined several different anti-a2 and anti-a3 antisera and have indeed found an anti-a2 and an anti-a3 antiserum which react with Hu IgG.

In conclusion, we have shown that Hu IgG molecules have at least one antigenic determinant of the rabbit a1 specificity. This a1 determinant in man was present in all 270 individuals tested, was found on at least 60% of the IgG molecules and was localised to the Fab fragment. Population studies with anti-a1 as well as with anti-a2 and anti-a3 purified rabbit antibodies reacting with human IgG should resolve the genetic basis for these determinants in man and indicate whether they are isotypic or allotypic antigenic determinants, that is, present in all individuals or segregated among the population as in rabbits. Studies with myeloma proteins of different classes and subgroups should be useful for the precise localisation and characterisation of these determinants in human immunoglobulins.

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Uptake of environmental antigens by the bursa of Fabricius

THE bursa of Fabricius of birds has an essential role as a central lymphoid organ for the differentiation of B lymphocytes^{1,2}. In addition, the bursa harbours immunocompetent B cells³ which are capable of local antibody production. Haemolytic plaque-forming cells have been observed in the bursa after introducing sheep red blood cells (SRBC) into the bursal duct⁴. Furthermore, epithelial cells of the bursal follicles have been shown to transport ferritin and India ink into the intercellular spaces of the underlying lymphoid tissue when the material has been introduced into the bursal lumen⁵. The bursal lumen opens through the bursal duct to the most caudal of the three cloacal chambers, the proctodeum, which is of ectodermal origin^{6,7}. The proctodeum leads to the outside through the anus and is closed externally by a strong sphincter muscle. The vent is surrounded on the outside by upper and lower anal lips. At the other end, the proctodeum opens into urodeum leading to the coprodeum and these form the cranial parts of the cloaca (Fig. 1a).

During surgical bursectomy, we observed that the anal lips of a chicken make peristaltic movements as if sucking something into the cloaca. When a liquid or suspension was applied to the external parts of the anus, it was rapidly taken inside by these movements. We hypothesised that at least part of the sucked material could be taken into the bursa and that this phenomenon probably serves as a trapping mechanism for environmental antigens. To test the hypothesis we examined the macroscopic and microscopic fate of material applied to the anal lips, and whether antigen administration by this mechanism leads to an antibody production.

We placed 0.05–0.1 ml volumes of barium sulphate on the anal lips of 6-week-old White Leghorn chicks, and visualised the intake by X-ray pictures. It seemed that this contrast medium was actively sucked through the proctodeum and the bursal duct into the bursa. In all, about 2 ml of the medium was taken into the bursa in 3 min (Fig. 1b–e). Only negligible amounts of the contrast medium appeared in the urodeum and coprodeum. When applying the medium for the X-ray pictures presented here, the chicks were kept in a position with the anus upwards. In this position, most of the contrast medium was evacuated normally and the same results were obtained when the medium was applied to the anal lips of chicks in the normal position—even though this was technically more difficult.

To study the microscopic distribution of the material sucked by the bursa, 0.1–0.5 ml of India ink (Pelikan 17 noir, Gunther

Wagner, Germany, dialysed overnight against physiological saline) was applied to the anal lips of 4-week-old chicks as described above. The chicks were anaesthetised with ether, and the bursae removed at appropriate intervals for microscopic examination. Two minutes after the application, no carbon was observed in the bursal tissues, 15 min after the application, some fine carbon particles occurred superficially in the follicle-associated epithelium and after 2 h the tracer had spread throughout the follicle-associated epithelium (Fig 2a). The cylindrical epithelium lining the bursal lumen and not associated with the follicles was almost always free of the carbon. Six hours after the application, fine and coarse carbon granules were observed in the central part of the follicular medulla (Fig 2b). These findings demonstrate that material available on the anal lips makes intimate contact with the lymphoid cells in the bursa so that antigenic sensitisation by this route is possible.

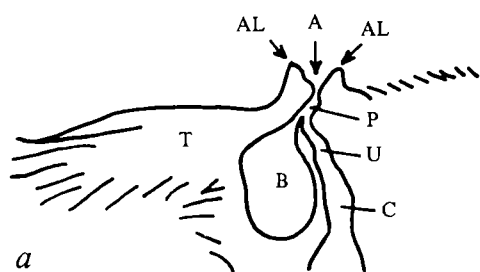


Fig 1 a, Schematic presentation of the three cloacal chambers and the bursa of Fabricius in the chicken. A, anus, AL, anal lips, P, proctodeum, U, urodeum, C, coprodeum, B, bursa of Fabricius, T, tail. b-e, X-ray visualisation of barium sulphate applied on the anal lips. About 2 ml of the medium was sucked into the bursa in 3 min. Only negligible amounts appeared in the urodeum and coprodeum.

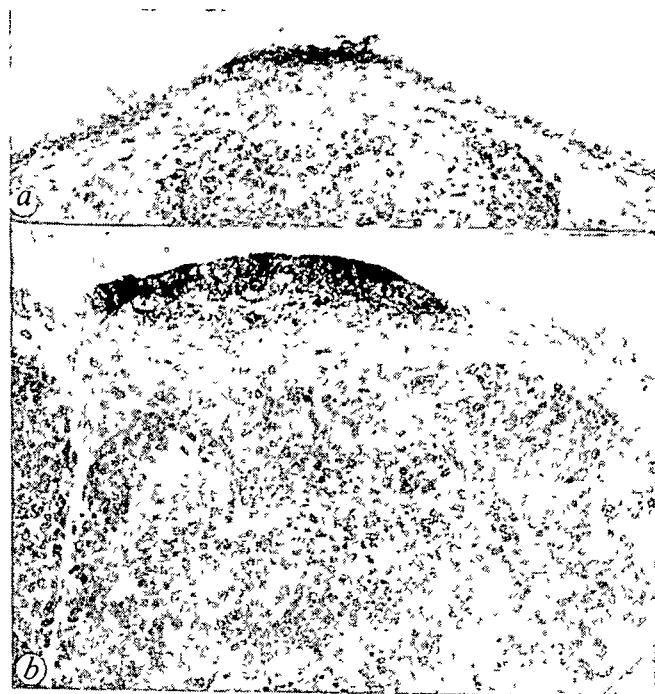


Fig 2 Bursal sections after application of dialysed India ink on the anal lips. a, 2 h after the application, carbon granules appear in the follicle-associated epithelium while the follicle is free of carbon, b, after 6 h, carbon has spread more widely and deeply into the follicle-associated epithelium, and carbon granules are observed also in the medulla of the follicle. Weak methylgreen nuclear staining ($\times 256$).

To examine whether the antigenic challenge through the bursa leads naturally to antibody production, 10^8 killed *Brucella abortus* organisms and 10^{10} SRBC in 0.1 ml of physiological saline were applied to the anal lips of 4-week-old chicks as in the experiments described previously. The procedure was repeated on six consecutive days. Blood samples from a wing vein were collected on the day after the final antigen administration. High serum antibody titres against a thymus-independent antigen³, *Brucella*, were observed, whereas serum antibody titres against the thymus-dependent SRBC were close to those found in unimmunised controls (Table 1). We interpret these findings to indicate that antigenic challenge *per bursam* leads to an effective antibody formation if no T-B-cell cooperation is required. This interpretation is supported by the vigorous antibody formation by transferred bursa cells against *Brucella* but not against SRBC in T and B-cell deficient recipients³.

On the basis of these findings, it is apparent that the immunological function of the bursa of Fabricius is not restricted to its inductive role in the B-cell differentiation but that it also has an important peripheral function. Furthermore, it is apparent that this peripheral function does not serve only local antibody production but leads to significant antibody titres in the blood. It remains to be established whether this antibody formation is restricted to that against thymus-independent antigens and whether it leads to formation of antibodies of different immuno-

Table 1 Serum antibody titres (mean \pm s.e.m.) after antigen application on the anal lips

Antigen*	No. of chickens	Log ₂ antibody titre†	
SRBC + <i>Brucella</i>	9	0.7 \pm 0.2	6.8 \pm 1.0
Saline	10	0.1 \pm 0.1	0.4 \pm 0.2

* 10^8 killed *Brucella abortus* organisms and 10^{10} SRBC in 0.1 ml were applied to the anal lips of 4-week-old chicks on six consecutive days.

†Serum antibody titres were determined from samples taken on the day following the final antigenic challenge, a microagglutination technique was used².

globulin classes in the same way as obtained after parenteral immunisation. It is also conceivable that constant presentation of environmental antigens in the newly-hatched period to the cells proliferating in the bursa leads to antigen-generated antibody diversity^{8,9}.

In conclusion, we suggest that a major function of bursa of Fabricius, in addition to its role as a central lymphoid organ, is to provide a chance for an early antigenic challenge by environmental antigens. The sucking function described here ideally serves this purpose.

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Ly antigens as markers for functionally distinct subpopulations of thymus-derived lymphocytes of the mouse

THYMUS-DERIVED lymphocytes (T cells) have several functions¹⁻³, and in some instances it appears that two subpopulations of T cells interact to amplify their response^{4,5}. But recognition of the diverse functions and interactions of T cells has not been matched by the development of techniques for identifying and separating the different T-cell subpopulations involved. The ability to do so would greatly aid study of the many roles of T cells. We report here experiments with mice that show that antisera to different Ly alloantigens can identify functionally-distinct subpopulations of T cells.

The *Ly-1* and *Ly-2* loci (chromosomes 19 and 6) specify alloantigens expressed exclusively and invariably on mouse T cells⁶. Each locus has alternative alleles that determine the T-cell surface antigens *Ly-1.1* and *Ly-1.2*, and *Ly-2.1* and *Ly-2.2*, respectively.

The four corresponding Ly antisera were produced by alloimmunisation with T cells as described⁷ and were absorbed with syngeneic thymocytes, if necessary, to remove thymocyte auto-antibody⁸. The mice used were of the B6 strain (*Ly-1.2*, *Ly-2.2*), and of B6 congenic partner strains with phenotypes *B6/Ly-1.1* and *B6/Ly-2.1*.

Table 1 shows the proportions of peripheral lymphocytes, and of cortisone-resistant thymocytes (CRT), lysed by optimal concentrations of *Thy-1* (formerly θ) antiserum,

and by these Ly antisera in the cytotoxicity assay with rabbit complement (C). Ly antigens are present only on T cells, and we shall assume that all T cells are *Thy-1*⁺ (that is they carry *Thy-1* antigen), we have therefore expressed the results in Table 1 as proportions of T cells (*Thy-1*⁺ cells) lysed by each Ly antiserum (% T) as well as proportions of the total population (abs = absolute).

The higher proportion of cells lysed by anti-*Ly-1.2* serum compared with anti-*Ly-2.2* serum was highly reproducible (Table 1) and may signify that there are more *Ly-1.2*⁺ T cells than *Ly-2.2*⁺ T cells among the peripheral T-cell population of B6 mice. This difference could also be explained, however, by differences in the constitution of the particular *Ly-1.2* and *Ly-2.2* antisera used (for example by different proportions of C-fixing antibody compared with other classes of antibody) and therefore more evidence is required on this point.

To determine whether T cells with different functions could be distinguished by characteristic Ly phenotypes, three functional assays for T cells were performed with cell populations pretreated with each Ly antiserum and C under essentially the same conditions as those of the C-dependent cytotoxicity assay⁹. We assayed (a) helper function of spleen cells from mice primed with sheep red blood cells (SRBC), (b) cell-mediated cytotoxicity (CMC) of non-adherent peri-

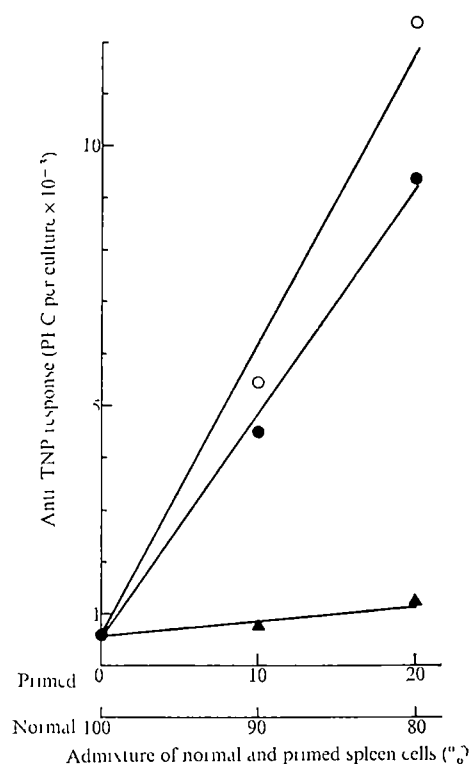


Fig 1 Effect of pretreatment with different anti-Ly sera and C on the helper function of spleen cells. Three mice were injected intravenously with 0.1 ml of 0.01% (v/v) SRBC. After 4 d their spleen cells were pooled and pretreated with Ly antisera and C (see below). Cultures were prepared containing these pretreated primed cells together with unprimed cells in various proportions (as indicated), maintaining a constant total 1×10^7 cells per culture. The cultures were sensitised with trinitrophenol (TNP)-conjugated SRBC and assayed 4 d later for TNP-specific PFC^{11,12}. For pretreatment, equal volumes of cells (5×10^6 ml⁻¹), antiserum (1/20) and complement (rabbit serum preabsorbed with mouse cells in the presence of EDTA) (1/6) were incubated in 5% CO₂ in air for 45 min at 37°C. These are the standard conditions for the C-dependent cytotoxicity assay⁹ (see Table 1). The diluent was Earle's balanced salt solution with 2.5% foetal bovine serum, 100 U ml⁻¹ penicillin, 100 µg ml⁻¹ streptomycin and 5×10^{-5} M 2-mercaptoethanol. A control for serological specificity (not shown) was substitution of *B6/Ly-1.1* (congenic) spleen cells for *B6/Ly-1.2* spleen cells, in this case pretreatment with anti-*Ly-1.2* serum and C gave no suppression of helper cell function. ○, Anti-*Ly-2.2*, ●, diluent only, ▲, anti-*Ly-1.2*.

Table 1 Cytotoxicity assays of Thy-1 and Ly antisera on various cell populations of B6 and B6/Ly-congenic mice

Serum anti-	Cells from	Titre on thymocytes (reciprocal)	Thymocytes	CRT		% Cells lysed* Lymph node cells		Spleen cells		NAPC	
				abs	% T	abs	% T	abs	% T	abs	% T
Thy-1 2	B6	10,240	>90	>90	100	66	100	32	100	39	100
Ly-1 2	B6	80	>90	75	83	57	86	27	84	29	74
Ly-2 2	B6	640	>90	55	61	43	60	21	65	22	56
Ly-1 1	B6/Ly-1 1	2,560	>90					26	81	30	77
Ly-2 1	B6/Ly-2 1	1,600	>90					10	31	8	21

CRT, cortisone-resistant thymocytes from mice given 1.5 mg of cortisone acetate intraperitoneally 24 h previously, abs, percentage of total population (absolute), % T, percentage of Thy-1⁺ cells present

* Figures corrected for background of dead cells by the formula $[(a-b)/b] \times 100$ where a is percentage cells viable in control (reciprocal) antiserum with C, or normal mouse serum (NMS) with C (always >90%), and b is percentage cells viable in cytotoxicity assay with antiserum and C

toneal cells (NAPC) from H-2-primed mice, and (c) graft-versus-host reactivity (GvH) of unprimed spleen cells

(a) The results of a typical assay of helper cell activity are shown in Fig 1. Helper function was virtually abolished by cytolysis of the SRBC-primed spleen cell population with anti-Ly-1 serum (specificity anti-Ly-1 2), whereas cytolysis with anti-Ly-2 serum (specificity anti-Ly-2 2) had no effect on helper function.

(b) In the CMC assay with peritoneal cells (NAPC) from allo-immunised (H-2-primed) mice the effect of the two Ly antisera was reversed. There was no suppression or only minimal suppression of CMC by pretreatment with anti-Ly-1 2 and C, whereas pretreatment with anti-Ly-2 2 and C strongly suppressed this ('killer cell') function¹⁰.

(c) The GvH (Simonsen) assay differs from the two preceding functional tests in that it involves initiation as well as implementation of the immune response. Both Ly-1 2 and Ly-2 2 antisera suppressed the GvH response of adult unprimed parental spleen cells inoculated into newborn F₁ hybrid recipients, as measured by Simonsen's spleen weight index.

Thus elimination of a proportion of T cells lysed by a particular Ly antiserum does not produce equivalent losses of different T-cell functions, and different Ly antisera eliminate T cells with different functions.

In these experiments the helper population was rich in Ly-1 and poor in Ly-2, and the reverse was the case with T cells responsible for CMC. The population involved in initiation and/or implementation of GvH evidently involves T cells expressing both Ly types, or more probably T cells of two Ly categories.

Table 2 Effect of pretreatment with anti-Ly sera and C on the cell-mediated cytotoxicity (CMC) function of non-adherent peritoneal cells (NAPC) from H-2-primed donors

NAPC* donors	Pretreatment†	CMC %I	
		Experiment 1	Experiment 2
Non-immune	Diluent only	0	0
Immune	Diluent only	50	76
Immune	NMS + C	64 (Standard)	78
Immune	Anti Ly-1 2 + C	65	67
Immune	Anti Ly-2 2 + C	28	30

* B6 donors received a single intraperitoneal injection of 10⁶ BALB/c (immune) or B6 syngeneic (non-immune control) spleen cells in Experiment 1 and multiple intraperitoneal injections of 5 × 10⁶ BALB/c (immune) or B6 syngeneic (non-immune) spleen cells in Experiment 2. NAPC were collected 5 d after an injection in Experiment 1 and 3 d after the last injection of four consecutive weekly injections in Experiment 2.

† See footnote Fig 1.

‡ ³H-proline CMC micro-assay^{13,14} in which the radioactivity of surviving attached monolayer target cells is measured. Washed NAPC (pretreated as in Fig 1, 1 × 10⁵ per well) were applied to a ³H-proline-labelled monolayer of BALB/c Meth A sarcoma cells (1 × 10³ per well) for 14 h. CMC % was calculated from the c.p.m. of the washed surviving monolayers after exposure to (a) Immune NAPC or (b) non-immune (control) NAPC, according to the formula $[1-(a/b)] \times 100\%$.

Table 3 Effect of pretreatment with different Ly antisera and C on the GvH capacity of spleen cells in the Simonsen assay¹⁵

Pretreatment of spleen cells†	Spleen indices of individual recipients†	Mean
None	1 98, 1 78, 2 03, 2 18 2 82, 2 21, 2 91, 3 28	2 44
Anti-Ly-1 2 + C	1 33, 1 67, 1 80, 1 08 0 95, 1 09, 1 14, 1 13 1 50, 1 21, 1 46, 1 75	1 27
Anti-Ly-2 2 + C	1 42, 1 42, 2 67, 1 57 1 81	1 64

* See legend to Fig 2.

† 1 × 10⁶ normal B6 spleen cells (pretreated as in Fig 2) were injected intraperitoneally into 1-5-d-old (B6 × BALB/c) F₁ hybrids. Spleen weight indices were calculated 9 d later according to Simonsen.

In addition to their implication with regard to the diversity of T cells, our findings suggest a means of isolating T-cell subsets with particular functions according to their Ly profiles. At the moment this can be said safely only of the B6 mouse with which these experiments were conducted, it remains to be seen whether the Ly profiles established for T-cell functions of this strain will prove to be representative of all mice.

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There's more to telecommunications . . .

THE Science Research Council (SRC) has recently come out with a report by one of its specialist subject panels which deserves a wide readership, both because it raises some fundamental issues about scientific research in universities and because the topic with which it deals—telecommunications—plays such an intimate part in everyday life. The report considers the SRC's own support for telecommunications research in universities and polytechnics, and does some plain speaking about the nature of the research itself and the environment in which it is carried out (*Telecommunications*, Science Research Council, State House, High Holborn, London WC1R 4TA).

First of all, the panel contends that the emphasis in university telecommunications research is in the wrong place. Basically long-term work on, for example, signal processing and systems studies is eschewed, the panel says, in favour of transmission and propagation studies: as a rough guide 15 out of 31 SRC research grants current in June 1974 were for work on transmission lines, waveguides, antennas and propagation. On the other hand only three grants were awarded for studies of communications networks.

The drift of the panel's recommendations, which the SRC is putting about for discussion and comment, is not hard to guess. It wants the SRC to make a gradual transfer of funds to the research topics it highlights as 'special areas'. It also thinks that "existing university effort [in telecommunications research], with one or two exceptions, is too thinly distributed to be effective for the broader inter-disciplinary research required . . ." In other words, the SRC is being asked to implement its formula of selectivity and concentration.

The panel adds a rider, however, to the effect that there should still be funds available for "individual workers" to research on "limited aspects" of telecommunications. It says, however, that it does not want to see unnecessary duplication of work done, for example, at its own Appleton Laboratory (formerly the Radio and Space Research Station).

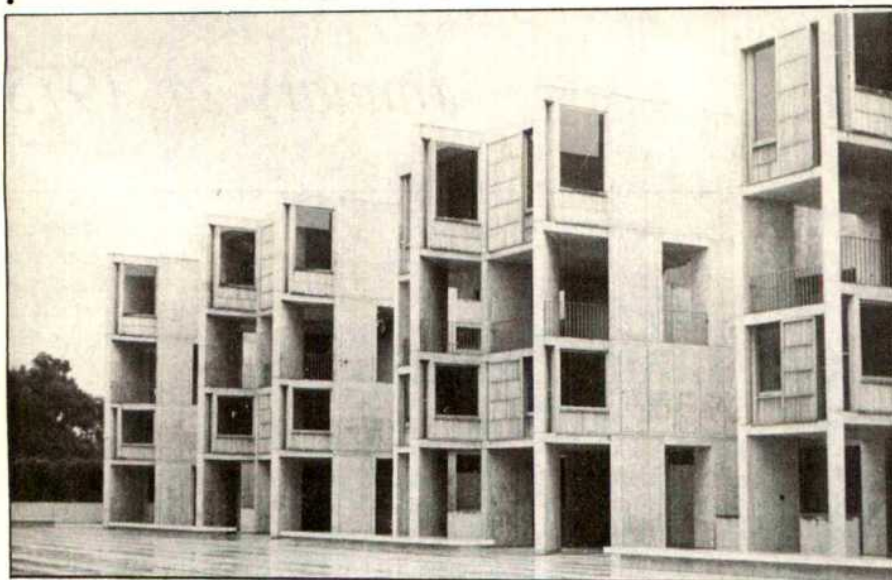
As far as research in universities in general is concerned there are two main questions that arise—whether the universities themselves should be thinking more along the lines of selectivity and concentration, unprompted by bodies like the SRC, in the light of the present stagnation of university funds, and whether postgraduate students would be better off if the doctrine of selectivity and concentration were practised more widely. If more universities are ever again set up in Britain one hopes desperately that they will not feel it

incumbent upon themselves to have, say, a geology department just because every university has to have one. There seems little doubt that the research student is better off in the long run in a department that is both large and broadly based—so that he emerges not as a diluted specialist but as a good specialist with a generalist aura about him. Interestingly, the report shows industry apparently facing in several directions over the usefulness of a PhD in telecommunications—some companies are adamant that the PhD training is irrelevant, whereas one says that "the best young members of its research staff have almost all received PhD training". Both views, however, probably crystallise the underlying frustration within companies that many good graduates do PhD courses before entering industry, and imply that it would be nice if that training created more advantages to the companies concerned. The SRC panel seems to deal sensitively with this kind of thinking.

Leaving university research aside, the panel's report is also important in that it spells out a catalogue of research that needs to be done in what has become an all-pervading aspect of modern life. At a time when broadcasting in Britain seems to be going through a rough patch, academics could well get their teeth into an examination of the pros and cons of broadcasting and cable systems for television, or take a fundamental look at the most effective way of using the frequency spectrum for radio, to name two of the panel's own examples. Then there are the possibilities opened up by the idea of integrated local telecommunications services—carrying telephone signals, for example, along with radio, television and other signals—and the desirability of refining conventional telephone systems to accommodate the special needs of groups like the old and the disabled.

The panel might well also have included a mention of the links between good telecommunications and the conservation of energy and other resources, though this is implicit in the idea of integrated local services. Even now a telephone conversation is often quicker and cheaper in energy than writing a letter, and projects like CEEFAX (which would provide instantly accessible news and other information on a domestic television set) are likely to have an impact on the present energy-intensive means of disseminating information through newspapers.

The SRC plainly thinks the universities have much to offer, and it can now only sit back and see if the appropriate response is forthcoming. □



Salk of human kindness

Armed with promises of financial support, 27 acres of prime building land donated by the citizens of San Diego and a strong conviction that science should be used for the good of man, Jonas Salk, an immunologist whose name became a household word when he developed the world's first polio vaccine, began in the late 1950s to plan a new venture. He wanted to establish a biological research centre dedicated "to contributing . . . to the health and well-being of man". The resulting Salk Institute for Biological Studies, now 11 years old, is a remarkable place. Colin Norman reports.

SET atop low cliffs overlooking the Pacific Ocean, just up the coast from the Scripps Institution of Oceanography and a stone's throw from the San Diego campus of the University of California, the institute probably ranks among the best known scientific establishments in the world. Salk's name alone is enough to ensure a good deal of publicity for the place, but it has also been pushed into the limelight through the works of one of its fellows, the late Jacob Bronowski, whose epic television series "The Ascent of Man" opened in North America this month. And, within the scientific community, the institute has already carved out a considerable reputation for itself in several areas of biomedical science.

Equally celebrated is the institute's stunning architecture. A striking concrete and glass design endowed with more than a touch of cubism, the structure is in fact so well known that the place has become something of a tourist attraction and the buildings have even been used on several occasions as a backdrop for fashion photographs. (Permission to film a battle scene in the central courtyard was, however, denied on the grounds that it would have been rather distracting for the scientists.) As well as attracting tourists and photographers, the buildings have also spawned reams of prose

in publications ranging from textbooks on modern architecture to *Time* magazine, the prize for which must surely go to the following florid description: "A spread of related buildings (conceived) to propitiate the gods of science even as the ancient Athenians built the Acropolis to keep their gods happy".

But how does the institute propitiate the gods of science in a manner different from other, less glamorous, research establishments? Salk's intention was to create an institute where research would be devoted primarily to health problems, but he also wanted to add an extra dimension of humanism to the place to ensure that the research is indeed, devoted to enhancing the well-being of man—biology with a conscience, Salk has called it.

In several respects, the institute has turned out much as its founder intended, but the grand design has altered a little over the years—"evolved is a better word", Salk insists—and there have recently been some significant changes in the way in which the institute operates. It has also run into its fair share of problems, not the least of which have been a chronic shortage of funds and, until recently, some discontentment among the younger researchers there.

Salk says that he first conceived the idea of founding a scientific institute in 1956, two years after his momentous

development of a killed polio virus vaccine. He was assisted in the planning by a number of prominent scientists, among whom the nuclear physicist Leo Szilard is said to have been influential, and his chief supporter was Basil O'Connor, the President of the National Foundation-March of Dimes (NFMD). The project began to take concrete shape in the early 1960s with the help of the architect Louis I. Kahn and financial backing from the NFMD (which also supported Salk's work on the polio vaccine), on a plot of land in the wealthy San Diego suburb of La Jolla. The first senior scientists were appointed in 1963.

The basic idea was to bring together a number of eminent scientists and give them tenure, freedom from teaching duties, a large amount of laboratory space, and it was hoped an adequate budget for their work. These so-called resident Fellows would provide overall scientific direction for the institute themselves, with the outside help of a panel of equally eminent non-resident Fellows who would meet once a year, advise on major new projects and on senior staff appointments.

The first resident Fellows reflected in large measure Salk's background in immunology and virology. Appointed in 1963 were the immunologists Melvin Cohn and Edwin Lennox, both of whom joined Salk from the Pasteur Institute, the virologist Renato Dulbecco, who moved down the coast from the California Institute of Technology (and who has since left to join the Imperial Cancer Research Fund in London, although he is still technically a resident Fellow of the Salk) and Jacob Bronowski, the mathematician, philosopher, broadcaster and humanist, who joined the Salk Institute from the British National Coal Board.

The next fellow appointed was Leslie Orgel who moved to the institute from the University of Cambridge in 1965 to continue his research into the chemical evolution of life. The Nobel Prizewinner Robert Holley came to La Jolla from Columbia University in 1968, and has since been working on the factors that control cell growth and division. And the latest senior appointment was Roger Guillemin, who moved from the Baylor College of Medicine in Houston to the Salk Institute in 1970, where he has continued his research into the control of the pituitary—work which recently led to the discovery of a new hormone produced in the hypothalamus, called somatostatin, which may be useful in the treatment of juvenile diabetes.

A common theme among those eight resident Fellows is that most of them have broad backgrounds—a factor which Salk says was deliberately sought when the appointments were being con-

sidered. Lennox, for example, was a nuclear physicist before he turned to immunology, Holley worked on nitrogen fixation and transfer RNA before he became interested in cell division, Orgel moved into prebiotic synthesis from inorganic chemistry, Bronowski started out as a mathematician, applied his skills to problems of evolution, developed a smokeless fuel, and was active in writing and broadcasting, and Salk has lately been engaged in writing on philosophy and ethics.

The non-resident Fellows, who meet in La Jolla in January each year to discuss the scientific affairs of the institute, include Nobel Prizewinners Jacques Monod, Gerald Edelman and Salvador Luria, and the President of MIT, Jerome Weisner. Although they play less of a role in the institute's affairs than they did during its early days, their input is still significant. Luria, for example, says that he often feels "more involved in decisions at the Salk than at MIT".

Until relatively recently, the scientific work of the Salk Institute was almost totally dominated by the research interests of its eight resident Fellows, each of whom heads a research group. In the early days, for example, much of the grant money flowing into the institute was channelled through the Fellows to members of their research group—a situation which led to some disenchantment on the part of younger scientists who wanted to pursue their own lines of research. Another cause of frustration was the fact that only the Fellows had tenured appointments; the other scientists were, in theory, less secure at the Salk than their colleagues were in the universities.

But a number of factors have recently altered both of those causes of disharmony, so that the Salk Institute now seems a relatively harmonious place to work in. Perhaps the most significant development has been the setting up of a number of independent research groups consisting mostly of younger scientists working in such fields as neurobiology, cancer, linguistics and reproductive biology. Their work is not tied to that of any of the Fellows, and according to one long-standing member of the institute, the result has been a great improvement in communications between the major research groups there. Another scientist put it more vividly: "a few years ago, nobody talked to anybody else; now the communication is excellent". One particular area which has recently benefited from such collaboration is that of cell division, where studies in three different laboratories at the Salk have recently converged to provide some clues to the factors which cause cells to grow and divide.

As for the question of tenure, last

year eight members of the institute were given the title of Associate Research Professor, and given conditions of tenure similar to those which pertain in the universities. For some of the younger scientists at the Salk, the fact that there is now demonstrably a career structure in the place could be an important factor in persuading them to stay there.

But the institute still has its problems. Like most centres of learning these days, it is short of funds, with the result that it has been difficult for the institute to move into major new areas of research. Another consequence is that the institute's work is now heavily concentrated in cancer research, largely because cancer money is relatively easy to come by compared with funds for other areas of science. A little over half of the institute's work is now concerned with cancer and related studies.

Salk points out, however, that since the institute is interested in "whatever seems to be in the forefront of advancing knowledge", cancer research is a natural area to be involved with, and it is also a good area for attracting bright young scientists.

The institute has also been forced recently to live much more off government funds than it used to. Last year, for example, some \$4.5 million of the \$7 million operating budget came from government grants and contracts. The rest came roughly in the form of a \$1 million grant from the National Foundation-March of Dimes, about \$1 million from other foundations, and \$0.5 million from private donations.

The financial affairs of the institute have, however, recently been placed on a firmer footing by the appointment of Frederic de Hoffmann as President and chief administrator. A nuclear physicist who worked on the Manhattan Project, de Hoffmann has, according to one Fellow of the institute, "brought a sense of financial responsibility" to the

place, and he is also credited with providing the impetus for many of the recent changes such as the establishment of more independent research groups and the extending of tenure to scientists below the rank of Fellow.

One measure of the increased financial stability is that the institute is hoping soon to raise sufficient money to begin a major new programme in neurobiology. Because plans for several large programmes had to be shelved when funding became tight in the early 1970s, some 35% of the laboratory space in the institute has never been occupied, and so the neurobiology programme, if it gets under way, will still leave the institute well short of its maximum capacity.

Another area in which there has been talk of expansion is the institute's human affairs programme. But that received a severe setback last August by the death from a heart attack of Jacob Bronowski, and it is yet to be determined how that side of the institute's activities will develop. Although Bronowski's work did not impinge much on the activities of most of the other scientists in the Salk Institute his range of interests clearly added an extra dimension to the place—a dimension which Salk says "will continue so long as I am here". A scientist closely connected with the institute suggested, however, that it has "never really managed to be a place where biology and the humanities were fused", chiefly because Bronowski's human affairs programme involved too few people. "A critical mass was never reached", he suggested, and there were consequently too few areas of overlap with the rest of the institute's members.

Be that as it may, the Salk Institute now seems to have entered a period of slow growth, paced chiefly by the availability of funds. After its first eleven years of evolution, it remains, in Luria's words, "overall, an exciting place". □

Some of the resident and non-resident Fellows of the Salk Institute. From left to right: Jacques Monod, Gerald Edelman, Melvin Cohn, Leslie Orgel, Robert Holley, the late Jacob Bronowski, Frederic de Hoffmann, Salvador Luria, Paul Berg, Edwin Lennox and Roger Guillemin.



international news

THERE used to be a standing joke in Washington that whenever Mr Nixon wanted to demonstrate that his besieged Administration was in control of events, he would announce a set of new policies for dealing with the energy crisis which were mostly so ineffectual that he usually succeeded in demonstrating just the reverse.

Although the energy policy announced last week by President Ford has run into many of the criticisms that greeted Nixon's plans, and although it stands virtually no chance of being approved by Congress, it at least takes a tougher stand on some of the more controversial issues. And it will also receive much more serious consideration than some of the proposals that have gone before.

Ford's plans, announced as part of the State of the Union message, fall into three parts. During the next three years, he wants to cut 2 million barrels of oil a day from imports, chiefly by employing a few moderate conservation measures, allowing energy prices to increase, and relaxing some environmental controls on the burning of coal. For the medium term, he has proposed a number of measures to increase domestic energy supplies in order to make the United States "invulnerable to economic disruption by foreign suppliers by 1985". In the longer term, Ford reckons that the United States can "develop [its] energy technology and resources so that [it] has the ability to supply a significant share of the energy needs of the free world by the end of this century".

Few people have quarrelled with the goals, but many have taken exception to the means. In particular, Ford's conservation and environmental proposals have drawn heavy sniping from Congress, and his plans for stepping up domestic energy supplies have met with doubts that they can be achieved, mixed with criticisms because they may be environmentally destructive. All in all, the chances that they will be approved on Capitol Hill look slim.

As far as the short term proposals are concerned, Ford has announced that he will put a tariff of \$3 a barrel on imported crude oil by April this year. He has the authority to do that himself, without the approval of Congress, and so unless Congress acts to stop him it will be done. In addition, Ford wants Congress to pass legislation to allow the prices of natural gas and domestic petroleum to increase, and he has asked

Ford spells out his energy plans

by Colin Norman, Washington



Ford: three-point plan

Congress to amend the Clean Air Act to allow power stations in polluted areas to switch from oil and gas to coal. Finally, to prevent oil companies from making excessive profits from the energy crisis—as they have been doing for the past two years—Ford wants Congress to agree to legislation which will put a heavy tax on profits not ploughed back into research and development or exploration.

The need for some swift action is obvious. Although energy consumption decreased during and immediately after the Arab oil embargo, it has recently begun to increase so that the United States is now importing about 7.3 million barrels of oil a day, compared with 6.5 million barrels immediately before the embargo. If no conservation measures are taken oil imports are predicted to increase to about 8 million barrels a day by the end of 1977.

But will Ford's proposals do the trick? As far as the consumption of gasoline is concerned, there is good reason to believe that they will not. The Administration estimates that a combination of the import duty and the deregulation of the price of domestically produced petroleum will add about 10 to 12 cents on the retail price of a gallon of gasoline. In the first nine

months of 1974, however, gasoline prices jumped by about 40% in some parts of the country but consumption has been increasing during the past three months by about 300,000 gallons a day. (It did, however, drop by some 3.4% early in 1974, although some of that may have been because people had to queue for hours to get any gasoline at all.

Nevertheless, the Administration predicts that the higher prices will decrease imports of oil by 1 million barrels per day in 1975 and 1.6 million in 1977, while conversion of oil-fired power stations to coal will save about 300,000 barrels a day of oil imports by 1977.

For the medium term, Ford has quietly dropped his predecessor's oft-proclaimed goal of energy independence by 1980, and he has adopted instead the much more realistic aim of reducing oil imports to between 3 million and 5 million barrels a day by 1985—a level which, he says, will preclude economic disruption from a sudden shutoff of foreign supplies. To achieve such a goal, he has proposed a programme to bring on line within the next 10 years 200 nuclear power plants, 250 major coal mines, 150 major coal-fired power plants, 30 new oil refineries, 20 plants to produce 'synthetic' fuel from coal gasification and liquefaction and from oil shale, "many thousand" oil wells, and the insulation of 18 million homes.

All that will require Congress to approve legislation which will expedite the licensing of new nuclear power plants, allow strip mining of large amounts of coal from the western United States, provide tax credits for insulation of houses, allow the leasing of tracts of land on the outer continental shelf for oil drilling, and extend the time period in which power plants must remove sulphur emissions. In addition, Ford has asked Congress to approve an amendment to the Clean Air Act which will freeze controls on automobile exhaust emissions at essentially the levels to be imposed on 1976 car models, and he has also asked for authority to purchase facilities for a national oil storage depot capable of holding 1,300 million barrels of crude oil.

That package of proposals is likely, however, to draw opposition from Congress on a number of counts, chief of which is the inherent retreat from a number of painfully conceived environmental goals. Consider, for example,

the proposal to delay automobile emission standards. Ford has proposed that strict emission controls which were to come into effect in 1976 should be put off until 1981, in exchange for which the automobile manufacturers have promised to improve by 40% the average gasoline consumption of new American cars by 1980. But Senator Edmund S. Muskie, who heads a key subcommittee which will consider the proposals, has already denounced it because it "trades public health for fuel economy".

Muskie and a number of other observers have pointed out that fuel consumption of 1975 car models was nearly 14% better than that of 1974 models, in spite of the fact that Detroit had to meet strict pollution control standards for the later models. They therefore point out that automobile manufacturers can meet the fuel economy goal without sacrificing environmental controls. Furthermore, a committee of the National Academy of Sciences reported last year that it could see no technical reason for relaxing the standards, and that the cost of meeting them is outweighed by the benefits.

The plans for expediting the nuclear licensing programme may also draw fire from Congress because there is a small but growing number of nuclear sceptics on Capitol Hill, and the Joint

Committee on Atomic Energy, which would normally shepherd such a bill through the Congress, has been considerably weakened by the retirement and defeat of nearly a third of its members. To help head off opposition to the nuclear programme, however, Ford has proposed increasing the budget for research on nuclear safety, waste management and safeguards by \$41 million next year.

A more fundamental question raised by Ford's proposals is whether the United States has the capacity to achieve even the redefined goal of energy independence. In the past few years, according to the Administration's own figures, electricity utility companies have scrapped or postponed 60% of their plans to build nuclear power plants and 30% of those for non-nuclear plants, and this at a time when Nixon was urging all-out expansion to meet the 1980 independence deadline.

The reason was lack of capital for investment, and so Ford last week proposed a number of measures to attract investment capital to the utilities, and to allow electricity rates to increase steeply. That is a prospect which many legislators will not relish explaining to their constituents when they come up for re-election in 1976.

Furthermore, a committee of the National Academy of Engineering pro-

duced a study last year which simply tallied all the measures that would have to be taken to meet the goal of zero oil imports by 1985, the total of which was so staggering that the committee concluded that it is highly unlikely that the goal could be achieved. Although the redefined goal will be easier to meet, there is nevertheless considerable doubt that the capacity can be established in time.

For the long term, Ford has reiterated the Administration's commitment to energy research and development, and has indicated that nuclear power is expected to play a central role in the energy mix towards the end of the century, by which time he hopes that the United States will be so flush with energy that it can export some to the rest of the world. Although he was not very specific about which technologies will get preferential treatment, a background statement distributed with Ford's message at least indicates that the breeder reactor may have slipped a little in the list of priorities. It used to be the number one energy programme in the Nixon Administration, and although the statement indicates that some means must be found to eke out uranium resources, it says that "the breeder reactor is only one such supply source" under consideration. □

THE Administration's plans for promoting the development of nuclear power in the United States were sharply rapped from both sides last week. Two groups of scientists, each replete with Nobel Prizewinners, traded statements in Washington urging, on the one hand, a greater commitment to nuclear power for meeting energy demands and, on the other, criticising the Administration for putting too many of its eggs in one basket.

Although such sentiments are far from new, seldom have they been placed in starker contrast.

The pro-nuclear statement, which was largely the work of the physicist Hans Bethe, was released at a press conference the day after Ford unveiled his energy plans. Signed by 34 eminent scientists, including eleven Nobel Prizewinners, the statement began: "We, as scientists and citizens of the United States, believe that the Republic is in the most serious situation since World War II", and it goes on to deplore the fact that long range energy plans are emerging too slowly.

The statement urged much greater commitment to the use of coal and nuclear power. "We can see no reasonable alternative to an increased use of nuclear power to satisfy our energy needs", it states, while "coal is irreplaceable as the basis of new synthetic

fuels to replace oil and natural gas". The statement also insists that although conservation is desirable, large cuts in consumption can be made only at the expense of jobs.

Asked why he drafted the statement, Bethe said that he had "felt for some years that nuclear energy was not getting enough emphasis", although he said that Ford's energy proposals—which were announced after the statement was written—are a great improvement. Among those who endorsed the statement were William O. Baker, President of Bell Laboratories, Harold Brown, President of CalTech, Joshua Lederberg of Stanford University, Franklin Long of Cornell University, Edward Purcell of Harvard University, Glenn Seaborg, former chairman of the US Atomic Energy Commission (AEC), Frederick Seitz, President of Rockefeller University, Edward Teller of Lawrence Livermore Laboratory, and Richard Wilson of Harvard University.

But critics of nuclear power have also taken exception to the Administration's nuclear plans, though from a different standpoint. On the day that Bethe released his statement, Ralph Nader sent a letter to President Ford urging him to "personally review the implications of dependence on nuclear power", citing "the unique and substantial hazards associated with the

massive amounts of radioactive materials that would inevitably be created by a full scale nuclear power program". The letter was endorsed by eight eminent scientists.

Nader's letter notes that nuclear accidents could endanger "our children and their children, for generations" and states that early enthusiasm for nuclear power has "been steadily eroded as the problems of catastrophic accidents, long-term waste disposal, and the specific hazards of plutonium have been more fully appreciated". In contrast to which, Bethe's statement says that nuclear critics "lack perspective as to the feasibility of non-nuclear power sources and the gravity of the fuel crisis", and expresses "confidence that technical ingenuity and care in operation can continue to improve the safety in all phases of the nuclear power program".

Although neither statement will have much impact on the Administration's plans, they do at least testify to the wide divergence of opinion within the scientific community on nuclear safety. And a heated exchange at the press conference between the chairman, Ralph Lapp, and Daniel Ford, a spokesman for a group which has played a leading role in opposition to nuclear power, confirmed that those opinions are very firmly held.

WORD is getting about that next year's federal grants for research in Canada will be greater than they have been for some years. How much greater no one will say until the official announcement is made (possibly in February), but they will probably increase at least a little in real terms.

Since 1975 will be the year of the new federal granting structure, this may do something to restore the flagging faith of Canadian scientists in their government's interest in science—flagging chiefly because of the steady decrease in research funds available relative to costs and inflation.

At the same time, student enrolment has risen steeply. In six years, starting with 1968, the total Canadian student population increased by about 30%. But in the same period, biology enrolments alone, for example, increased almost 130%. And in eastern Canadian universities the increases were even greater—45% for total enrolment and 190% for biology. During the same period there was an increase of 70% in the number of full-time biology teachers.

But neither operating budgets nor research grants have kept pace. One chairman of a biology department reported a doubled student enrolment in five years coupled with a decline of 20% in his operating budget.

Since 1969-70, the total increase in parliamentary appropriations to the National Research Council (NRC) of Canada for research grants and graduate scholarships has been only 7%. Yet during the same period the cost of research is estimated to have risen by about 50%, and inflation in the cost of scientific equipment and materials has been even greater.

Thus the effective investment in research covered by the NRC grants has actually been reduced by more than a third. The Medical Research Council did little better.

Pronouncements by the government that reorganisation of the granting councils is going to take place this year have done little to reassure the scientists. Last February's Speech from the Throne, in which the announcements were made, "indicates only a concern for procedure and administration", said Dr J. A. Morrison, Director of McMaster University's Institute for Materials Research. "If the present trends continue, there may not be any academic research to administer within five years."

Now it looks as though the government intends to modify its policy somewhat. Late last year, the government approved a supplementary grant of \$2.5 million for the Medical Research Council. And in a House of Commons session last November, C. M. Drury, Minister of State for Science and Tech-

Canadian government ponders MOSST's mission

from David Spurgeon, Ottawa

nology and former Treasury Board President, indicated that the situation was being studied sympathetically. Given the increase in prices and inflation, he acknowledged, "it would be necessary to raise the amounts [of grants] for university research."

Another minister, Hugn Faulkner, the Secretary of State whose department is responsible for grants for the social sciences and the humanities, tried to reassure academics on the reorganisation of the granting bodies in a speech to the Association of Universities and Colleges of Canada; this is another matter that has been vexing them recently.

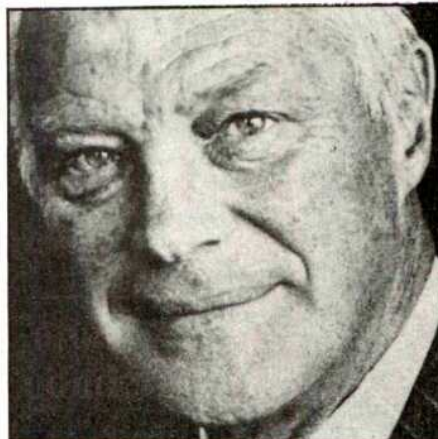
"No radical changes in the granting policies and practices are sought," he said. "... I can assure you that in drafting the bill, which will be tabled in the course of this session of Parliament [probably not before April or May] every possible effort is being made to take into account the fundamental concerns of the academic community."

The most effective guarantee of the acceptability and effectiveness of these councils will lie in the quality of those appointed to them, the minister said, and "the government intends to give its closest attention to this matter before making its final decisions."

Such reassurances were needed because, altogether, 1974 was not a very good year for instilling in scientists confidence in the government's approach to science policy. Part of the problem was that everybody was trying to figure out what the new Ministry of State for Science and Technology (MOSST) was up to. Conceived in controversy and born in discord (in 1971) the MOSST seemed to be suffering from an identity crisis.

The case was admirably—if rather

Drury: last-ditch effort.



critically—set out in a report published by another government creation, the Science Council of Canada, which has the independence to hire consultants, who do studies for it which it then publishes. This one was called *Knowledge, Power and Public Policy* (Science Council of Canada Background Study No. 31, Information Canada, Ottawa). Its authors were Peter Aucoin, a political scientist, and Richard D. French, a science historian.

The study examined the concept of the ministries of state, which were established with the authority of a 1970 bill to be responsible for designated policy fields not encompassed within the jurisdiction of any single existing government portfolio. The two such ministries studied were science and technology, and urban affairs.

"The ministers of state", say Aucoin and French, "would be faced with a novel task. The organisations that would serve them would not be departments in any traditional sense, but rather ministries whose initiatives would inevitably and consistently involve the responsibilities of other ministries. Fundamental to the notion of a ministry of state is the idea that the activities of research and policy analysis can provide an adequate basis for successful policy formulation and co-ordination. The logic underlying such a ministry derives from the 'knowledge-is-power' hypothesis: namely, that research, information and analysis will carry the day in Cabinet and Cabinet committees against the traditional sources of political and bureaucratic power."

They do not see the concept as a great success so far. Concerning the two ministries studied, they say: "Neither Ministry of State can be said to have had the kind of policy success that was envisaged when they were created." And concerning the MOSST: "The performance of the ministry in relation to the scientific and technological community can hardly be considered a success to date [early 1974]."

Aucoin and French conclude that "the most promising strategy for the MOSST may well be a more modest, more pragmatic, more incrementalist, and less visible role than heretofore", one with a non-threatening service posture rather than a directive one.

There are signs that the suggestion is being heeded. Some see Mr Drury and his deputy as having been sent to make a last-ditch effort to clear up the ministry's problems, in an attempt to determine whether it really can survive or not. And Drury is reported to have commented privately, "If you want to know what will happen to the MOSST, read the Aucoin-French report and use your common-sense." □

THE next move has now been made in the discussions on the desirability and safety of certain types of experiment aimed at producing microorganisms containing new combinations of genetic material. According to the Working Party set up last August by the Advisory Board for the Research Councils under the chairmanship of Lord Ashby, the potential hazards inherent in many of the new techniques for manipulating the genetic make-up of bacteria can be sufficiently minimised to allow the work to continue.

The problem was brought to the attention of the public by a group of American scientists who had pioneered these techniques. A committee of the National Academy of Science under the chairmanship of Professor Paul Berg proposed a moratorium on several lines of research: the construction of new plasmids containing combinations of virulence or drug resistance genes not found naturally, and the transfer of such plasmids into organisms in which they do not already occur naturally; and the linkage of DNA from tumour viruses to plasmids or other viral DNA. Much of the danger from these experiments stems from the fact that the bacterium most commonly used as host is the common gut bacterium *Escherichia coli*, which might be the means of disseminating the new genetic combinations amongst humans with unpredictable results.

Conventionally trained microbiologists and bacteriologists have often regarded with some horror the cavalier way in which molecular biologists and biochemists have tended to treat the organisms with which they work, and can perhaps be forgiven for regarding the current concern over bacteriological hazards with some sense of *déjà vu*. Their answer to the problems posed by possible novel organisms is that with proper precautions these bacteria can be contained in the same way that dangerous pathogens have been successfully contained in the past.

While recognising that the cases are not exactly parallel, the Working Party in its report comes down firmly in favour of the view that proper precautions can indeed reduce the risks from experiments in genetic manipulation to an acceptable level. They recommend that all those working with the new techniques should be trained in dealing with pathogenic bacteria and should have access to expert advice on the precautions necessary in any given case. All laboratories contemplating such work should be properly equipped and very hazardous experiments should only be carried out in special laboratories.

Certain simple and relatively inexpen-

sive precautions, such as no smoking, eating or drinking in the laboratory, wearing gowns and gloves which are removed before leaving and the sterilisation before disposal of all contaminated material, should suffice in most cases. More sophisticated procedures are available for dealing with material such as tumour viruses where the risks are potentially greater. There are also ingenious ways in which bacteria can be to some extent "disarmed" by muta-

Round Britain



tions which do not allow them to grow at above a certain temperature or without some rare growth factor.

Other recommendations include the epidemiological monitoring of all those working with the new techniques, special precautions to be taken when dealing with large-scale experiments and investigation into systems which might prove less hazardous than the commonly used *E. coli*-drug resistant plasmid combination.

The report should provide a timely basis for discussion at the conference called by Professor Berg and scheduled to be held next month.

● The daring young man on the flying machine is Dr Magnus Pyke, Secretary of the British Association for the Advancement of Science, currently to be seen on British television, advertising the appearance of a new partwork about science. Actually his machine amounts to nothing more than a pair of roller skates and a handful of bricks, so nobody seriously expects Pyke to fly. The aim is simply to demonstrate the principle which causes a jet to move—equal and opposite reaction stuff which

comes into play when Pyke throws the bricks in one direction and rolls off in another. The BA Secretary, who always makes entertaining radio or television, says the demo fits neatly his function as an explainer of scientific thought to a mass audience. So it's an important roll.

● The recent death in a London hospital of a patient suspected to be suffering from the virus disease Lassa fever highlights the growing problem of importing exotic and often dangerous diseases into Britain.

Ever since its discovery in Nigeria in 1969, Lassa fever has been viewed with trepidation by health workers. The four epidemics which have occurred in West Africa since 1969 have all been marked by a high death rate among hospitalised cases and by a high risk of infection by close contact, which puts nurses, doctors and relatives caring for the patients at great risk from virus-laden blood, urine and other body secretions.

There have been several cases of Lassa fever imported into Britain, the most recent being earlier this month, when a doctor returning from Kano in Nigeria, died of Lassa fever in the London Hospital for Tropical Diseases two days after his arrival in the UK. As with many tropical diseases, the main problem is a correct diagnosis, especially in Britain. Lassa fever in particular starts with a slowly mounting fever followed by headache, backache and nausea, and unless the doctor is aware that the patient has recently arrived from West Africa, where the disease is endemic, it is almost impossible to diagnose.

● GALLOPING inflation coupled with the slump in capital values of investments has finally forced the closure of one of the oldest established biomedical laboratories in Britain, the laboratories of the Lister Institute of Preventive Medicine in Chelsea Bridge Road, London.

An extra £300,000 a year at current prices would be needed to keep the laboratories in business. But at the end of last year, it was announced that it had been impossible to secure sufficient new funds to prevent the laboratories from closing in 1975.

Warning notes have been sounded over the past few years and the research effort had already been reduced to a level which the Governing Body regarded as tolerable only in the very short term. The laboratories in Chelsea Bridge Road were opened in 1898 and have been in continuous use for the 77 years of their existence. Over the years the institute has made important contributions to the understanding of infectious diseases and parasitology, as well as of basic chemistry and biochemistry.

More than just preparing

from Vera Rich, London

THE launching of the two-man spacecraft, Soyuz 17, and its link-up with the orbiting space station, Salyut 4, should not be considered simply as a preparation for the forthcoming Soyuz-Apollo project. So said Major-General Georgii Beregovoi, Head of the Cosmonaut Training Programme, at a press conference given after the successful link-up. On the contrary, he says, the joint Soviet-USA missions will merely be a part of the Soyuz programme, "a chance to broaden the range of tasks which can be solved using the Soyuz craft".

Although this comment may be interpreted as an echo of the confidence which the successful link-up has evoked in the Soviet space planners after a year notable for its setbacks, there is more to it than simply post-launch euphoria. The primary aim of the current mission, said Beregovoi, is to test out a new control and life-support system. According to the TASS data, this involves approximately normal atmospheric pressure and temperature in the cabin, representing a return to standard Soviet procedure after Soyuz

16, which with its reduced cabin pressure and increased oxygen content was a compromise with US practice.

The control system referred to is presumably the new autonomic navigation system, based on the use of ionic sensors and permitting the station to be orientated with respect to the Sun, Moon and planets in various regimes of orbital flight, and which also includes a device for orientating the spacecraft with respect to the earth in conditions of minimum illumination above the night side of the planet.

Nevertheless, even if Soyuz 17 is not a direct preparation for the Soviet-US mission, its success or otherwise must surely affect the confidence of the space planners of both nations in the outcome of the joint project. □

The Shtern case

The case of Dr Mikhail Shtern, formerly Director and Senior Consultant in the polyclinic of the Vinnitsa Provincial Endocrinological Health Centre has, during the course of pre-trial investigations and the trial itself, caused considerable reaction in the West, which has not passed unnoticed by the Soviet authorities. In the pre-trial investigations, the charges against Dr Shtern ranged from dissi-

dence to the murder of child patients. Apparently as a result of the protests, and also because it proved impossible to find witnesses to substantiate the murder charge, when the case finally came to trial the only charges Dr Shtern faced and those for which he was duly sentenced were those of "swindling" (charging for medical treatment) and "the taking of bribes".

Although these are clearly less serious charges than murder (or, in a Soviet context, dissidence), they seem intended to take Dr Shtern's case outside the competence of such organisations as Amnesty International which undertake to deal with cases of "prisoners of conscience". In the past 18 months, how every, there have been several cases of would-be emigrants to Israel facing criminal charges and, in Dr Shtern's case it was admitted on May 29, 1974 by the Procurator of the Investigations Department, V. Kravchenko that the preparation of an accusation against Shtern was connected with the desire of his family to emigrate to Israel. Accordingly, those who have been campaigning for Dr Shtern have shown no signs of being put off by the circumstances of his conviction. New letters of protest are being prepared, seeking a reversal of the verdict and sentence. □

THE Israeli Government has set up a Ministerial Committee for Science and Technology to tackle problems of research and development, and to determine whether activities in these fields are in line with the general policy of the government.

The scientific community in Israel feels that the establishment of the new committee is long overdue. Scientific and technological activities in the country have expanded extensively during the past five years; mainly because of governmental support its share in total civilian research and development and education investments was 69% in the fiscal year of 1973-74. This share increases when investments in defence research and development are included, since these are totally covered by the government.

The overall investment in research and development and higher education—both civilian and defence—reached a total sum of IL 1,500 million. There is, however, growing public criticism because of what is felt to be a lack of central policy in allocating government and public funds to achieve more direct and positive results. For instance, it is felt here that universities—which are private institutions—are supported too heavily by the state, whereas elementary and secondary schools suffer from lack of funds and teachers. It is also felt that a good deal of the

money spent on civilian research and development yield few benefits to the country in these trying days.

Israel is blessed with a 'brain influx', an inflow of professional manpower. An exceptional phenomenon for a small and developing country, this influx consists of local university graduates, of Israeli researchers who return home after working abroad for several years (mostly in the USA), and of

New Israeli science committee

from Kipai Pines, Jerusalem

scientist-immigrants from North and South America, Europe and lately from the Soviet Union.

Alas, the blessing might become a misfortune if this manpower is not used to best advantage. A central policy is needed and it is hoped that the new Ministerial Committee for Science and Technology will also tackle this problem.

The committee's tasks include: (1) ratification of the general policy of the state for the advancement of scientific research and technological development and the application of

them to meet the economic and social needs of the country; (2) deciding the goals of the state's budget in the fields of research and development, and its priorities; (3) defining inter-ministerial procedures which will assure coordination of all government operations in science and technology; (4) rectifying any changes in the organisational layout of the research carried out in government laboratories; (5) confirming appointments of Chief Scientists in state ministries; (6) confirming any other functions in the fields of science and technology which the committee will think fit.

To stimulate the committee and to give emphasis to inter-ministerial problems, it is composed of ministers whose ministries engage in scientific and technological functions: The Prime Minister (chairman), a minister without portfolio (vice-chairman), and the ministers of finance, defence, health, education and culture, agriculture, commerce and industry, interior, housing and transportation. The coordinator of this committee is the Director-General of the National Council for Research and Development, Dr Eliezer Tal.

● Professor Saadia Amiel, one of Israel's leading atomic scientists, has been appointed to the strategic planning division of the Defence Ministry, according to a report in the *Jerusalem Post*.

North American diary

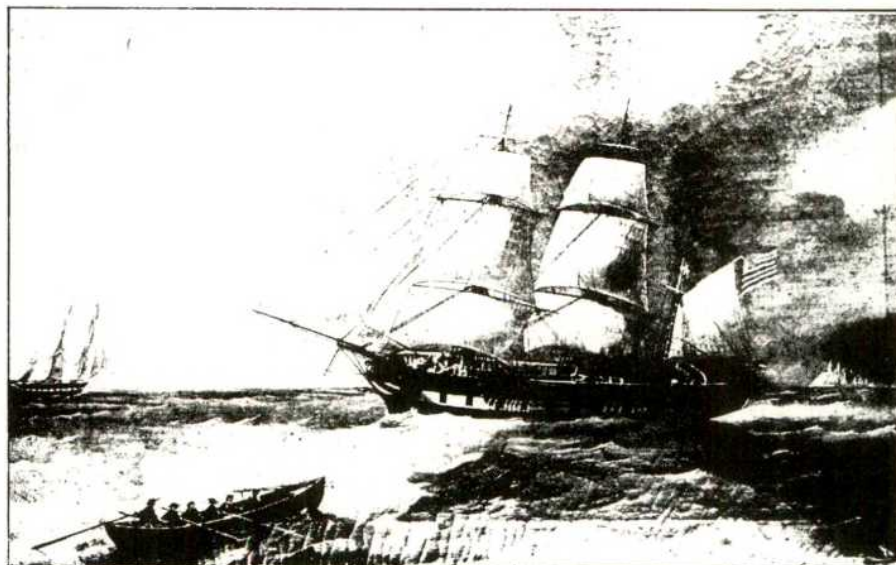
from Angela Croome

THIS month off an island in Hudson's Bay and about 2,000 miles from anywhere else an American adventurer plans to bore a hole through some six feet of sea-ice and with heavy lifting equipment hoik to the surface a New Bedford whaler that sank there 100 years ago. The assumption is that the hull would remain intact during this rough-and-ready recovery but if not, Alexander Byron, the New Bedford restaurateur behind the scheme, is equally confident of cobbling it together on the spot and sailing the ship back to its Massachusetts home port. All this in the polar night with temperatures down to -22°C .

He is offering similar caesarean treatment for the remains of the two Knight expedition ships of 1719, the wrecks of which have also been found on the bottom of the Eastend harbour of Marble Island. These were a British sloop and frigate chartered by the Hudson's Bay Company through the enthusiasm of its lately retired and nonagenarian 'governor' of North American depots, Sir James Knight, who was convinced of a river of gold at the end of the equally mythical North-west passage to the Pacific. The expedition got no further than Hudson's Bay, lost both ships in a violent storm and perished lingeringly after three years cut off from the mainland on Marble Island. The Knight expedition is one of the most pitiful tales in the whole gamut of polar exploration, and there would be unique archaeological and expeditionary interest in the actual remains of this early polar voyage. Most of this evidence is bound to be lost by the recovery methods proposed at present. One can imagine the outcry there would be in Britain if a 250-year-old expedition ship and its contents were to be rudely winched from the sea bed without regard to conservation, recording or any other archaeological techniques.

Not so in Canada—although two distinct licences have to be obtained from government departments before any project may be pursued in the Northern Territories. Admiration of the old North American virtue of "can-do" still, it seems, outweighs the value put on the epic past.

● The campuses are taking the lead in concern for the energy environment in a way that one looks for in vain outside America. While British government offices have only recently introduced a 60°F heat limit, all the professors' houses I visited in the USA and Canada this winter had their temperatures set at something less than



A New Bedford whaler of the type that Alexander Byron may fish up.

60° , and had been so at least since the beginning of last winter.

Walking is becoming quite fashionable at Princeton, Harvard, McMaster, Sarah Lawrence and other suitable centres though a nasty mugging of a sociologist's wife in Cambridge recently has discouraged movement on foot after dark. Bicycles are widely replacing cars on campuses too—sometimes with rather grisly results because American academics are wedded to the idea that cycling is *sportif* and that a bicycle is not 'traffic'. The common regulations of lighting and flow direction are scarcely observed and it is a disconcerting experience indeed when driving at night to meet an oncoming cyclist, head well down, with no lights and on the wrong side of the road.

This approach contrasts strongly with that of the motorist. The reduction in traffic deaths during last winter's fuel crisis was so remarkable that a speed limit of 55 miles an hour has been adopted permanently for all roads in the USA. The amazing thing to a European is that everyone conforms, and in the most orderly and disciplined fashion. The massive overpowered machines, still gobbling gas at a rate of about 15 miles to the gallon, progress on the expressways in a well spaced and stately stream at an average $52\frac{1}{2}$ miles an hour. Driving is emphatically no longer a blood-sport in the United States—as it is (one realises anew) for Europeans, especially the British, and, indeed, for Canadians. There is no nonsense with a national speed limit or courtesy between drivers on the six lanes of the Queen Elizabeth Highway, supposedly the world's most travelled road, which connects Toronto to the industrial heartland of the United States.

● Cholesterol is the other general campus preoccupation. While women

and children tuck in as ever to butter and eggs, the middle-aged husband resolutely sticks to dietary margarine and a one-egg-a-week regime. And, of course, he 'jogs'—typically a mile a day and in appropriate dress. The whole thing is taken so earnestly, with or without medical evidence of enhanced cholesterol levels, that one wonders whether the cholesterol scare is a subtle conspiracy by Women's Lib to chastise the profligate male.

● Vasectomy—an operation only really popular in England among policemen apparently—is both widely performed and discussed. "Did I know many men who had had the operation?" Well, I do now. Perhaps this is another reflection of the leadership of the campuses in environmental concern.

● But there is a freemasonry among early morning exercisers that has much to recommend it. A Washington journalist who shares a taste for tennis rather than jogging with one of the country's most dedicated astrophysical experimenters, found himself the repository of speculation about why the scientific press neglected the amazing results emerging from the new generation of super-large radio interferometers. The 90-mile baseline proposed for this type of work when—and now if—Jodrell Bank's out-station at Meifod, Wales, were built is piffling in comparison with the power of interferometer systems using baselines which rely on telescopes continents apart. Such an arrangement is already in use synchronising telescope observations in the USA and the Soviet Union. The next stage, a Moon-Earth link-up, would give a baseline 250,000 miles long and an analytical power in proportion. Already the infrastructure of cosmic processes in undreamt-of detail is rolling off the computers on which this instrumental approach depends. □

Message to the stars

from Ian Ridpath

MANKIND'S first deliberate message to other civilisations among the stars has long since left our planetary system, leaving behind it on Earth certain rumblings about the way in which it was done. The media, both popular and scientific, outside the USA have turned such a deaf ear to this momentous event that the adjacent diagram, transmitted by the slower methods that remain conventional on Earth, may be the first detailed description of it to reach many parts of the community.

The 1,679 part message was transmitted in 169 seconds from the 1,000-foot Arecibo radio telescope in Puerto Rico. The frequency used was 2,380 MHz (wavelength 12.6 cm)—not one of the standard lines suggested for interstellar communication, but instead the top end of Arecibo's radar astronomy facility that is being used for projects such as the detailed mapping of Venus.

Writing in *Nature* for October 5, 1973, Frank Drake and Carl Sagan of Cornell University noted that the telescope's few capability would allow it to communicate with an identical instrument anywhere in the Galaxy. The Arecibo message, transmitted after a re-dedication ceremony following the resurfacing of the Arecibo arch, can only be seen as an attempt to demonstrate the truth of their proposition.

The key to the message is that it breaks down into a grid, 23 characters by 73. The diagram shows the picture that can be built up from it. Reading from top right (a tricky start) the pictogram describes in binary form the numbers one to ten as a kind of lesson to establish the language of the following message. Below this top row of numbers is a group displaying the atomic numbers of hydrogen, carbon, nitrogen, oxygen and phosphorous.

Next, the message uses this information to describe the molecular components of DNA and on lines 32 to 46 actually depicts DNA's double-helix structure. The central core is the number 4,000 million—roughly the number of characters in the genetic code.

All this adds up to a description of the chemical basis of terrestrial life: next comes a cryptic human (Ned Kelly in gumboots?) with an indication of his height to the right (14 wavelengths of the transmission) and on the left the approximate number of the human population (4,000 million again).

On the next line is a sketch of the solar system, with below it a representation of the Arecibo telescope itself, pointing downwards to a number that roughly describes its diameter.

This interstellar IQ test was devised

by members of the National Astronomy and Ionosphere Center, of which Arecibo is part. Radio astronomer Frank Drake of Cornell University, which runs Arecibo, says that the contents of the message were arrived at after exhaustive discussions, and after being 'market tested' to see how easy it was to decipher. Some people, doubtless familiar with the workings of the originators' minds, managed to unravel most of it. But a spot check in the *Nature* office revealed no intelligences (native or alien) that could decipher it. Are interstellar IQ tests culture fair?

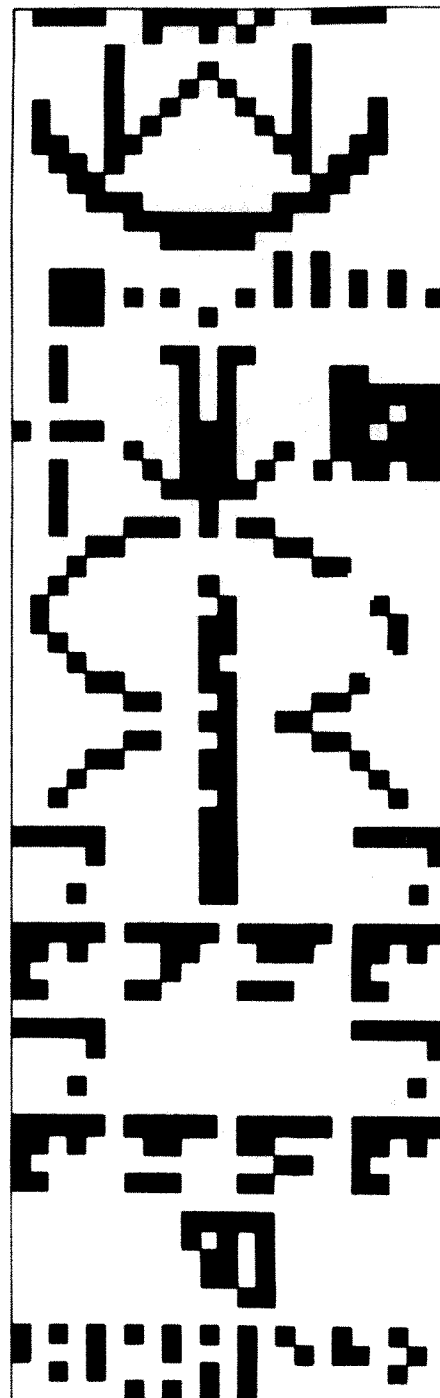
The message was beamed at the globular cluster M13, which is 24,000 light years away. At that distance the radio telescope beam just covers the cluster's 300,000 stars. Astronomer Carl Sagan estimates that there is a one in two chance that there will be a civilisation there to receive it. The signal frequency was modulated to correct for the motion of the Earth in space, although other radio astronomers have pointed out that just such a Doppler shift on an interstellar signal would give valuable information about the orbit of the sending planet and its axial spin, as well as its size.

The single transmission of such a message cannot be regarded as a very serious attempt at interstellar communication; more likely, it will serve as an example to boost the funding chances of those who wish to listen for similar messages coming to us from other civilisations. No prior news of the Arecibo message was given, even to delegates who discussed interstellar communications at the previous month's International Astronautical Federation meeting in Amsterdam.

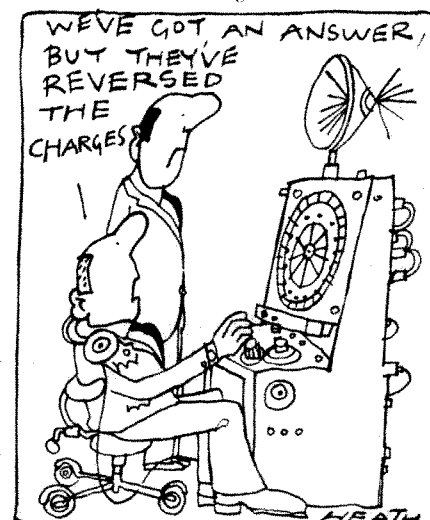
Yet at the 1971 International Meeting on Communication with Extraterrestrial Intelligence (CETI) the delegates concluded that such undertakings were best done "by representatives of the whole of mankind".

Drake now says that he did not consider the Arecibo message a major enough event to require international cooperation. But Tony Lawton, a member of the CETI standing committee of the International Academy of Astronautics, thinks that it has established "a very nasty precedent" and is frankly surprised that it has been done at all without prior international discussion and approval. He says that "now there is nothing to stop any nation from transmitting any signal, anywhere, to any spot it chooses. A little warning, a little consultation, would have been better."

The critics still have time to influence matters for the future. The Arecibo message is planned to be repeated when telescope time permits, although no dates have yet been chosen.



Above: the message. Below: answer?



Courtesy The Sunday Times

news and views

G-wizardry at Dallas

from John Faulkner

ASTROPHYSICISTS experience biennial urges to congregate in the Texas Symposia on Relativistic Astrophysics (irreverently, Gee-whizz meetings). Recently, five hundred participants assembled in Dallas where from 16–20 December almost fifty scheduled speakers and many more in so-called workshops, summarised progress since the last meeting (*Nature*, **241**, 169; 1973). Necessarily incomplete impressions follow.

Cosmology opened the proceedings. Reeves (SEP-CEN, Saclay), noting the embarrassingly large interstellar deuterium abundance, remarked "Anybody who wants to believe in the big bang producing a closed universe had better invent a way of making deuterium; and that's not easy, I can tell you". Colgate (University of New Mexico) suggested supernova shock waves as a site, but the general impression of a severe restraint remained.

Gunn echoed this theme in his State of the Universe message (traditionally reserved for Caltech and its satellites). Gunn and colleagues have assembled many arguments (stellar, nuclear, inferred densities, and so on) pointing to an open Universe (see *Astrophys. J.*, **194**, 543; 1974). He and Oke, with seventeen new redshift-magnitude points for $z > 0.25$, produced a "classical test" which disconcerted many. Tinsley (University of Texas) later presented spectroscopic evidence for giant-star dominated ellipticals. The implied evolutionary dimming necessitates reductions in observed values of the Sandage variable q_0 by 1 to 1.5 or more. Thus emerged a large, negative value for the true q_0 ; alternatively, a large positive value for Einstein's disinherited Λ , the cosmological constant. When Fowler (Caltech) arose to demand if this were so, Gunn retreated to the sanctuary of probable errors. Perhaps cosmologists should always state "These are the opinions upon which I shall base my facts".

Ostriker (Princeton University) supported the density estimates when discussing halos of galaxies. Galactic disk stability requires a massive halo. Elsewhere, masses from mass-luminosity ratios increase with scale up to the 'virial masses' of clusters. New data obtained with Spinrad reveal extended red halos around edge-on spiral galaxies; the now respectable virial masses still fail to close the universe.

The observations of neutral currents in weak interactions have had dramatic effects on supernova theory. Schramm (University of Chicago) summarised implications for collapsing iron-nickel core models (descending from the work of Fowler and Hoyle). Freedman's suggestion that neutrino scattering cross-sections will scale as the square of atomic number may be the key permitting central collapse to neutron stars of black holes, while producing mantle ejection. Arnett (University of Illinois) has followed evolution from well understood helium burning to the hydrodynamic phase of neutron-star formation. Wilson (Lawrence Livermore Laboratory) and Bruenn (University of Florida) found shock waves in collapse calculations. Whether black hole formation or nucleosynthetic eruption would ensue was unresolved. Later Weinberg (Harvard University) presented a virtuoso account of the furore in physics and astrophysical consequences. Neutral currents, charmonium and a possible unification of strong, weak and gravitational forces at large energies were tantalisingly dangled before a mesmerised audience.

The mysterious gamma-ray bursts were discussed by Strong (Los Alamos) and Cline (Goddard). In one doubly observed event Vela detectors caught only the tail-end; earlier onset data should thus be regarded with caution. Ruderman (Columbia University) regaled listeners with a scintillating review of the flood of unconstrained theories, more numerous than the events themselves, involving black holes, neutron stars, antimatter, white holes, novae, ablating relativistic dust grains. "Nothing is too wonderful to be true, but there is not yet much else to support such a host of clever inventions."

Developments in relativity theory were reviewed by Ellis (University of Cape Town) and Trautman (Warsaw). Ellis emphasised that Bianchi (spatially homogeneous but anisotropic) cosmologies can have very different singularity

properties from those usually considered. Matter can originate in spatially inhomogeneous regions, cross prediction horizons, and end up in spatially homogeneous regions. Some charmingly named universes (for example, "Whimper-bang-whimper") await discovery; it was noted with evident relish that the steady state begins not with a bang but a whimper. Teukolsky (Cornell University) reviewed black hole perturbations, where Chandrasekhar has developed some unifying formalism. Practical gravitational radiation was almost conspicuous by its absence (a noted observational characteristic?). The anti-Weber climate (*Nature*, **250**, 287; 1973) has had curious backlash effects on other work involving gravitational radiation. Spectroscopy of HZ29 showing evidence for the predicted mass transfer was first dismissed by an organiser as the work of self-evident cranks. Hawking (University of Cambridge) penitently confessed that black holes were white hot, emitting through quantum effects, radiation which makes small holes ($\lesssim 4 \times 10^{14}$ g) evaporate in less than the age of the Universe.

X-ray day dawned. A promise by Giacconi (Harvard University) that the meeting would now become interesting, was amply fulfilled after his talk when Pounds (University of Leicester) showed Cen X-3 data from the UK-5 satellite with a strong dip at phase 0.62 in each binary period. This was regarded as excellent support for the extended Pringle wake model which Giacconi had presented. The day ended with mad scrambles between two simultaneous X-ray workshops involving no fewer than 38 speakers.

Strittmatter (University of Arizona) was permitted 25 minutes to cover QSOs (how are the mighty red shifted!). After suggesting that much of the effort on BL Lac had been misdirected, he discussed the increasing evidence for multiple absorption redshifts and line-locking (for example CIV in 3C191) agreeing with Scargle's predictions. A new $z=3.2$ QSO was announced which, like OH471, appears neutral or reddish. It reopens the question of selection effects once more and emphasises the importance of working systematically through Hazard's suitcase-full of unbiased accurate positions. Small scale radio structure was described by Cohen (Caltech). Apparent linear 'expansion'

velocities up to $\sim 10c$ are seen, and in 3C273 three independently varying components simulating motion can be ruled out. Four or more components are required for the 'theatre marquee' effect to work, but the existence of 'expansions' only is a puzzle.

The organisers, perhaps with the misguided intent of reserving the finest vintages till last, arranged three of the most important papers for the final chaotic afternoon. The Brans-Dicke theory received two almost mortal blows. Hill (University of Arizona) finds the solar oblateness at a time when confusing excess brightness is absent to be lower than Dicke's value by more than an order of magnitude. It agrees with expectations for a uniformly rotating Sun to within 25%. Consequently, Mercury's perihelion shift has a negligible contribution from a quadrupole gravitational field and supports Einstein over Brans-Dicke.

One fascinating by-product emerged: old Sol is breathing (pulsating seems too strong a term) in a number of modes including the gravest, ~ 54 min (geophysicists note!). Fomalont (NRAO, Green Bank) reported results of the microwave radiation bending from three QSOs with angular positions close to the Sun's path in April. The result, 1.015 ± 0.011 times the Einstein prediction, is not consistent with a scalar coupling constant $\lesssim 23$. Finally, Taylor (MIT) announced that the binary pulsar 1913+16 is indeed showing apsidal motion of 4.0 ± 1.5 degree yr^{-1} , consistent with general relativity and suggesting two compact components of ~ 1.3 solar masses each.

The meeting was a great success, if at times in spite of the arrangements by the 19-strong organising committee. I thank those many speakers who, guided no doubt by self-preservation, provided summaries of their talks.

least some of this material would seem confirmed the dating is not quite so clear. The geological sequence in the Hadar area seems well worked out but the fact that this fossil material was not found *in situ* but was scattered over a considerable area of the surface has led the researchers to express some doubt over the exact position of its original location within the sequence. Thus the "niveau à hominidés" designation in the geological column is followed by a query (?).

In October, 1974 further hominid remains were reported from the Hadar area. These remains, consisting of four jaw fragments, include a complete maxillary arcade with only the incisors and one canine missing. The first report of this material, at a press conference in Addis Ababa on October 25 (see *The Times*, page 6; October 26, 1974), included the following statement:

"These specimens clearly exhibit traits which must be considered as indicative of the genus *Homo*. Taken together, they represent the most complete remains of this genus from anywhere in the world at a very ancient time.

"All previous theories of the origin of the lineage which leads to modern man must now be totally revised. We must throw out many theories and consider the possibility that man's origins go back to well over four million years."

These are rather strong statements and may be examined on several levels.

First, although the reported potassium/argon date of 3.01 ± 0.25 Myr for a basalt in the Hadar area may well be valid this fossil material, as with the 1973 specimens, was not recovered *in situ* but was collected on the surface. Thus, the specimens' relationship to the dated level cannot be known with complete certainty. In an area such as the Awash ephemeral stream activity can move material over considerable distances and the absolute dating of surface finds must be viewed with some scepticism. Second, no morphological or metrical data have been presented so that the attribution of this material to the genus *Homo* cannot be fully evaluated. It should be pointed out, however, that the available photographs show certain features of the canines, premolars and molars which may not be entirely consistent with current definitions of that genus. Third, the statement that "all previous theories" of hominine origins must now be revised in the light of this material is simply untrue. The results of earlier field work at East Rudolf, based on material found *in situ* and in a well-dated context, have already supported an early origin of the genus *Homo*.

The final point is not, however, the

Ethiopian fossil hominids

from a Correspondent

WITHIN the last decade and a half field work in east Africa has had an extremely important impact on all aspects of early man studies. The initial impetus for east African field studies came of course from Olduvai Gorge but subsequently successful field programmes at East Rudolf in northern Kenya and in the Omo River area of southern Ethiopia have been no less important and productive. In late 1973 a new area was added to the east African inventory of fossil man sites. This new area on the Hadar River, a tributary of the Awash River, in eastern Ethiopia has produced, to date, a total of nine fossil hominid specimens.

Geological and palaeontological studies in Plio-Pleistocene deposits in this area have been in progress since 1967. These studies have resulted in a brief report (Taieb, Coppens, Johanson, and Kalb, *C.r. hebd. Séanc. Acad. Sci. Paris*, 275, August 16, 1972) of geological sequences and palaeontological remains from several sites in the Awash area. Formations at Haoua-Lédi and at Leadu have been closely correlated, on the basis of faunal remains, with deposits in the Omo River valley dated to between 2-3 Myr. Deposits at Hadar may be a little older and have been faunally correlated with the lower levels at Omo, perhaps, in absolute terms, at about 4 Myr. This latter region yielded, in late 1973, five hominid fragments: an associated right distal femur and proximal tibia, proximal left and right femora and a left

temporal bone.

A full description of this material has not yet been published but a brief report (Taieb, Johanson, Coppens, Bonafille and Kalb, *C.r. hebd. Séanc. Acad. Sci. Paris*, 279; August 26, 1974) mentions that the femoral neck is flattened anteroposteriorly and is oval in section; the distal femur, said to be "très humaine", has an elongated and flattened external condyle and a deep intertrochanteric fossa. The authors, in stressing the very small size of this post-cranial material, conclude that the height of this individual from Hadar was less than that postulated for one of the individuals from Sterkfontein. Most estimates of the height of STS 14, for example, based on reconstructed femoral length, do not exceed $4\frac{1}{2}$ foot. Perhaps the most interesting and significant feature of the distal femoral fragment is not mentioned in the text but is apparent from the accompanying photograph. It seems clear that the natural anatomical orientation of this femur would include a relatively high degree of shaft obliquity. This angulation, which would permit the knees to approximate the mid-line during upright stance, is an important hominid characteristic and distinguishes the femora of this group from those of other primates. Although this material was not formally attributed to any taxa the morphology of the temporal bone, which is highly pneumatized, was said to resemble that of the robust australopithecines.

Although the hominid nature of at

ultimate or intrinsic scientific value of these fossils; the data are at present inadequate to make such an evaluation. The point is that questions of taxonomic attribution, phylogenetic position and, ultimately, scientific importance must follow upon careful comparative studies and complete anatomical and metrical evaluation. Such a reversal of priorities, with its consequent and inevitable de-emphasis on necessary laboratory procedures is completely inconsistent with current scientific method and theory.

Stagger to deceive

by David Davies

THE problem of detecting and identifying underground nuclear weapons tests has been around for nearly twenty years. In 1958 there were high hopes that seismic techniques would prove adequate to monitor tests down to a few kilotons without any territorial intrusion. A year later these hopes were dashed when the big-hole theory was wheeled out by the American weapons laboratories—fire an explosion in large enough a cavity and its seismic signal is almost negligible. In the early sixties hopes rose and fell as the Americans, British and Russians played, inconclusively, with numbers of on-site inspections and black boxes. In the late sixties hopes began to rise as seismic methods improved and a new technique was developed based on the relative excitation of surface and body waves. In the early seventies hopes rose to a peak when there was talk on an underground test ban but there was widespread disillusion when the nature of the ban (on explosions above 150 kilotons from a date in 1976) became clear.

Now a new problem emerges. In this week's *Nature* (page 242) Kolar and Pruvost report for the first time in the open literature on techniques that could be adapted by a country wishing to evade a strict ban.

It has been widely accepted for many years that a test fired while the Earth was ringing from a really major earthquake (of the sort that occurs only once or twice a year) would be impossible to detect. This presents a rather risky prospect to the determined evader; a more measured scenario in which the evader fired at a time of his own choosing would obviously be more desirable. Yet to do this some way had to be found to increase the excitation of surface waves relative to body waves. This could be done, argue Kolar and Pruvost, by firing a multiplicity of shots, one of which would be the device under test.



The dots are the locations of presumed 'Plowshare' explosions. The triangles are Soviet test sites.

The relative excitation of surface and body waves is not actually changed by this technique, but since the techniques for measuring the excitations are relatively crude (necessarily; signal-to-noise ratio is frequently no greater than unity) it is possible to deceive the measuring process. The dominant period of a body wave is 1 second and the rules for determining excitation specify a measuring interval of three or four seconds so the apparent magnitude from a string of explosions over many seconds is of necessity low. On the other hand surface waves have a dominant period of 20 seconds, so provided the time delay from first to last explosion is kept smaller than about 10 seconds, all surface waves add in phase. Thus the apparent enhancement.

Many other precautions will have to be taken to avoid satellite and ground monitoring, but Kolar and Pruvost believe that these can be satisfied and thus that there will be serious doubt if not complete misreading in the monitoring country.

Should such ideas for deception be surfaced, revealing a mistrust of Soviet intentions? Most certainly; they have been around in the grey area between secrecy and openness for several years, and it is now necessary to take them seriously so that in any future debate on test-ban technology there will be no surprises. It will not escape the reader that the authors work for a laboratory charged with research into nuclear weapons and therefore presumably with a certain interest in forestalling any total ban on weapons testing. But no

doubt many seismologists elsewhere with a different point of view will now try to crack this new problem.

● In a related field, the figure shows the enormous extent of Soviet 'Plowshare' nuclear explosive activity. Each point represents an event reported in the open seismic listings which can reasonably be taken to be an explosion for some peaceful purpose. Yields vary from a kiloton or less to more than 100 kilotons, and the sparse literature from the Soviet Union suggests uses ranging from canal-building to oil field engineering.

Sizing the RNA tumour virus genome

from Benjamin Lewin

WHEN RNA is extracted from the C-type RNA tumour viruses, the major components sediment at 60–70S and 4–5S. After denaturation by heating or by treatment with DMSO, 60–70S RNA sediments in neutral gradients at 30–40S. The apparent $10\text{--}12 \times 10^6$ daltons of the 60–70S RNA thus seem to comprise subunits of about 3×10^6 daltons; given the uncertainty inherent in this determination of molecular weights, there might be two, three, or four subunits in the 60–70S complex. A question that has remained unaltered for some time is whether the subunits in a virion all are identical (so the genome would be polyploid) or whether they carry different sequences (giving a haploid genome). The genetic

information contained in a single subunit would be sufficient to code only for about 300,000 daltons of protein, which compares with the total weight of known viral proteins of about 3,650,000 daltons.

The presence of two types of subunit in 30–40S preparations of Rous sarcoma virus (RSV) RNA was first reported by Duesberg and Vogt (*Proc. natn. Acad. Sci. U.S.A.*, **67**, 1673–1680; 1970) when they electrophoresed the RNA on gels; these are the more slowly migrating (larger) *a* subunit and the smaller *b* subunit. The size of the class *a* subunits is in the range $2.4\text{--}3.4 \times 10^6$ daltons and that of the *b* subunits is about $2.2\text{--}2.9 \times 10^6$ daltons (Duesberg and Vogt; *J. Virol.*, **12**, 595–599; 1973). But Martin and Duesberg (*Virology*, **47**, 494–497; 1972) then showed that the 60–70S RNA extracted from cloned RSV particles derived from clonal populations of infected cells carry only the *a* subunit. In a further report, Duesberg and Vogt (*Virology*, **54**, 207–219; 1973) showed that the *a* subunit of cloned Schmidt–Ruppin strain of RSV is slightly larger than the *a* subunit of the cloned Prague RSV strain.

Different (noncloned) strains of avian sarcoma virus have different ratios of *a/b* subunits. Avian leukaemia virus particles, however, contain only *b* subunits. Since the sarcoma virus can transform chick fibroblasts whereas the leukaemia virus is unable to do so, this observation led to the concept that the *a* subunit may carry information necessary to accomplish transformation and that this sequence is missing from the leukaemia virus genome. Consistent with this idea was the observation of Duesberg and Vogt that transformation-defective variants of avian sarcoma virus all possess only the class *b* size of subunit. The derivation of *b* subunits from *a* subunits might therefore be explained by the occurrence of a deletion in the *a* subunit which removes information essential for transformation. If all the subunits in either size class are identical, then a single event would be sufficient to generate the defective viruses; but if the subunits represent different sequences, presumably a deletion would have to take place in each type of molecule in order to generate the size change between sarcoma and leukaemia virus.

Two approaches have been taken to determine the complexity of the RNA genomes and these have yielded contradictory results. Chemical analysis suggests a polyploid structure for the avian RNA virus genome. After digesting the RNA of the Schmidt–Ruppin strain of Rous sarcoma virus with T1 ribonuclease and separating the oligonucleotides on two-dimensional gels, Billeter, Parsons and Coffin (*Proc.*



A hundred years ago

THE ZODIACAL LIGHT.—On the evening of Sunday last, the 24th inst., a surprisingly bright display of this as yet problematical phenomenon was exhibited. There was a repetition on the following evening, but in a less favourable sky. The light had the usual yellowish or pale lemon tinge of the more notable exhibitions in these latitudes. The axis of the light appeared to pass λ Piscium, and the vaguely-defined apex was situate somewhere about 19 Arietis, but it was not possible to locate it with anything like precision. The light was broad and of a deeper, perhaps, ruddy tint near the horizon. The display to which we have adverted, excelled in brightness any that has been witnessed in the neighbourhood of London for many years. It appears very probable that opportunities for favourable application of the spectroscope may be afforded in the dark evenings of the present and following months.

From *Nature*, **11**, 249, January 28, 1874

natn. Acad. Sci. U.S.A., **71**, 3560–3564; 1974) obtained roughly twice the number of spots generated by 28S rRNA. By determining the molar yield of 11 of these spots, they were able to suggest a complexity for the viral genome of about $3.4 \pm 0.9 \times 10^6$ daltons. A similar estimate was made by Quade, Smith and Nichols (*Virology*, **61**, 287–291; 1974) for the RNA of the Prague strain of RSV (3.3×10^6 daltons). These estimates would suggest that the genome is polyploid, since the sequence complexity of the viral RNA is about the length of a single 30–40S subunit molecule.

That *b* subunits are derived by a deletion of *a* subunits is suggested by the chemical and hybridisation analyses reported by Lai, Duesberg, Horst and Vogt (*Proc. natn. Acad. Sci. U.S.A.*, **70**, 2266–2270; 1973). Comparing the T1 ribonuclease digests of the RNAs of avian sarcoma viruses and transformation-defective derivatives showed that all the large spots of the *b* subunit are found in the digest of the *a* subunit; but the *a* molecules contain one or two additional spots not found in the *b* digest. The labelled cDNA prepared from the *a* subunit hybridises with about 70% of the *a* RNA when saturation is reached; it hybridises with 60–65% of the *b* RNA, a result which would be consistent with the omission from the *b* RNA of a sequence present in the *a* RNA. Neiman *et al.* (*J. Virol.*, **13**, 837–846; 1974) have compared the

RNA genome of the Prague strain of RSV with a spontaneous transformation-defective mutant derived from it by hybridising these viral RNAs with excess chicken DNA of normal embryos and sarcomas. The transformation-defective strain seems to lack a sequence present in the parent sarcoma virus.

Hybridisation experiments have been used also to measure the complexity of the 60–70S RNA of RSV. When labelled cDNA prepared from the viral genome is annealed with an excess of RNA, the reaction rate depends upon the complexity of the RNA. Taylor *et al.* (*J. molec. Biol.*, **84**, 217–221; 1974) reported a sequence complexity of 9.3×10^6 daltons, which corresponds to about three different subunits if each subunit is about 3×10^6 daltons. This clearly suggests a haploid genome. No reconciliation between the chemical and hybridisation analyses is at present apparent.

What is responsible for maintaining the association of subunits in the 60–70S complex? The denaturation maps recently constructed by Mangel, Delius and Duesberg (*Proc. natn. Acad. Sci. U.S.A.*, **71**, 4541–4545; 1974) suggest that hydrogen bonding at several locations may be implicated. The T4 gene 32-protein binds cooperatively to single stranded nucleic acid and (mildly) denatures both 60–70S and 30–40S preparations. The molecules treated with 32-protein can then be visualised by electron microscopy. In the presence of 32-protein, 30–40S RNA is linear, appears fully coated with protein (thus losing the extensive secondary structure seen in the absence of 32-protein), and has a maximum length (some molecules are shorter, presumably due to degradation) of 2.75×10^6 daltons. The complex of 32-protein with 60–70S RNA consists of several linear molecules held together at different sites, with four to eight free ends per complex. Most of the RNA is coated with protein. The average length is 6×10^6 daltons, which would suggest that the complex includes only two subunits.

Manganese can grow rapidly

from Peter J. Smith

FERROMANGANESE nodules and crusts typically grow on the ocean floor at rates of 0.1–1.0 cm per million years and have Fe/Mn ratios of 0.5–2.0. But Scott *et al.* (*Geophys. Res. Lett.*, **1**, 355; 1974) now report that they have found a manganese oxide (MnO₂) crust with a much higher growth rate, an abnormally low iron concentration and unusual trace element characteristics. The newly discovered crust is thus

atypical but apart from demonstrating just how fast manganese can accumulate under the right circumstances, it also appears to be indicative of hydrothermal processes along active ridge crests as recently proposed in more general terms by Lister (*Eos*, **55**, 740; 1974) and others.

The crust sample in question, which is a 4.2 cm thick deposit of birnessite with a trace of todorokite, was dredged from the median valley of the mid-Atlantic ridge about 5 km from the ridge axis at 26° N. The first hint of its anomalous nature came from a radiochemical analysis of uranium series isotopes. Deep sea manganese deposits usually contain quantities of ^{230}Th and ^{231}Pa far in excess of those required for equilibrium with the parent uranium isotopes, indicating that thorium and protactinium have been taken up from the sea water during the process of manganese accumulation. The decay of these excess isotopes may then be used to determine the rate at which the manganese was produced. But the sample analysed by Scott and her colleagues turned out to be depleted in ^{230}Th and ^{231}Pa ; the manganese accumulation rate had therefore to be measured from the growth of the isotopes towards equilibrium with their uranium parents.

The rates thus determined were, respectively, 13.0 and 25.0 cm per million years, which are held to be in "reasonably good agreement" but which compare with accumulation rates of only 0.1–0.5 cm per million years for nearby samples of 'normal' manganese. This disparity explains in part the unusual trace element composition of the Scott sample, for the concentrations of trace metals removed from sea water are generally the lower the more rapidly the manganese grows. Once the accumulation rate had been determined, therefore, the low observed concentrations of Cu, Ni and Co were hardly unexpected. However, as Ku and Glasby (*Geochim. Cosmochim. Acta*, **36**, 699; 1972) noted, this phenomenon does not apply to uranium, presumably because uranium is incorporated into the manganese in a different way.

Low trace metal concentrations, rapid growth rates and high degrees of fractionation of Mn from Fe are characteristic of both hydrothermal deposits and deposits remobilised from reduced sediments. But what makes a hydrothermal origin the more likely for the Scott sample is the fact that the Mu/Fe ratios of 360–3,600 (the Fe content is as low as 0.01% in places) are much higher than those found in diagenetic continental margin nodules. Other evidence supports the same view, but is more circumstantial. For example, bottom photographs show that the site from which the Scott sample

was dredged is essentially sediment-free; thermal measurements indicate a sharp 0.14° C increase in bottom water temperature over the site; and there is a local magnetic low which could reflect hydrothermal alteration of magnetic minerals in the pillow lavas. Taking all this evidence together, Scott *et al.* conclude that the MnO_2 crust was deposited by a hydrothermal spring in the mid-Atlantic ridge's median valley.

Non-coding regions of messenger RNA

from Pamela Hamlyn

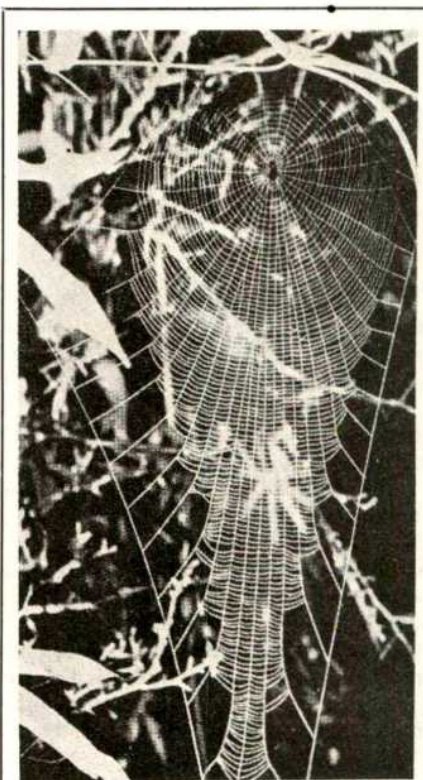
ALL purified messenger RNAs isolated so far have been shown to contain considerably more nucleotides than are required to code for their particular protein. Interest in these untranslated sequences has been centred round the possibility that they may be the site of interactions related to translational control of protein synthesis—a mechanism for which there is much circumstantial but little direct evidence. Part of this non-coding region is accounted for by the poly(A) sequence found in most eukaryotic mRNAs. Despite intensive research, the results of which have often been summarised in these pages, the function of this sequence remains obscure. Current research has shown that the poly(A) region becomes shorter as the messenger 'ages', suggesting that the length of the poly(A) region provides information about the number of rounds of translation possible before degradation of the messenger.

With these ideas in mind, Jeffrey and Brawerman (*Biochemistry*, **13**, 4633; 1974) compared the length of the poly(A) region in messengers from rapidly dividing undifferentiated cells (mouse sarcoma) with globin mRNA from rabbit reticulocytes—highly differentiated cells which do not divide. They confirmed that the poly(A) region becomes shorter with age but suggest that a terminal size is reached. Digestion of polysomes with ribonuclease yields poly(A)–protein complexes, and the authors were able to show that in mouse sarcoma these complexes become smaller and more heterogeneous with time, whereas poly(A)–protein complexes from polysomes synthesising rabbit globin are much more homogeneous. Unfortunately, these studies provide little new information, and consequently the authors have not been able to further elucidate the role of poly(A).

Results of research into the non-poly(A), non-coding regions of messenger RNAs are limited and seemingly contradictory. Nichols and Eiden (*Biochemistry*, **13**, 4629; 1974) have

reasoned that sequences next to the poly(A) region of mRNA might be recognition sites for poly(A) synthetases or else represent termination signals for transcription by RNA polymerase (poly(A) is added post-transcriptionally). They speculate that this would result in a region of homology in the sequences next to poly(A) throughout a population of messenger RNAs.

To investigate this, Nichols and Eiden have prepared ^{32}P -labelled polyosomal RNA from L cells, then digested it with RNase T_1 (an enzyme specific for guanylate residues) and, using poly(U) affinity chromatography, have isolated fragments which contain poly(A) and a few bases at their 5' ends. Sequencing of these bases showed that there was no particular sequence common to all (or even a large proportion) of the messenger RNAs, although more than 50% of the total mRNA molecules contained a single pyrimidine residue next to the poly(A). The authors conclude that there is no recognition site common to all mRNAs in terms of nucleotide sequence.



Web of immature *Eustala* (?) sp. from a forest on the Pacific coast of Columbia. W. G. Eberhard (*J. nat. Hist.*, **9**, 93–106; 1975) describes the construction of this web and an even more elongated one made by *Scoloderus* sp., also in Columbia. Both webs bear a remarkable resemblance to a ladder-like web made by a New Guinean araneid spider and described by Robinson and Robinson (*J. nat. Hist.*, **6**, 687–694; 1972).

Proudfoot and Brownlee (*Nature*, **252**, 361; 1974) have sequenced the 3' end of purified rabbit β -globin mRNA next to the poly(A) region and compared it to the sequence of bases in the same position in mouse immunoglobulin light chain mRNA (Milstein *et al.*, *Nature*, **252**, 354; 1974). They did not sequence the RNA directly but transcribed it into DNA using *E. coli* DNA polymerase I and deoxytriphosphates, one of which was labelled with ^{32}P .

Comparison of the two sequenced regions of each messenger showed that two sequences (7 and 4 bases long) were common to both messengers. As well as this sequence homology Proudfoot and Brownlee suggest that structural homology exists since both the light chain and globin 3' ends can be drawn with loops which are identically positioned and almost the same size in the two mRNAs. Most of the homologous sequence is probably in the unpaired region.

The apparent contradiction between these authors' results and those of Nichols and Eiden will probably disappear as the sequences of the 3' ends of more messengers become available.

Classical relativity in an up-to-date context

from W. H. McCrea

THERE has recently appeared a substantial paper by F. Salzman "Gravitational Field of a Freely Moving Mass" (*Il Nuovo Cimento*, **24B**, 157-188; 1974); the treatment is intended to be that of standard general relativity, howbeit with emphasis on the dynamical rather than the geometrical point of view, and the application is mainly to the case of a Schwarzschild mass. In days when there is so much interest in the gravitational and dynamical properties of black holes, such a study of intimately related problems would be expected to be particularly timely and significant. Indeed, the surprising thing is that the problems have not long ago been fully investigated. Nevertheless, while the paper is of much interest in an exploratory way, it seems that definitive results are scarcely yet achieved.

Some of the immediate background theory is quoted from the well-known books by C. Møller and by L. D. Landau and E. M. Lifshitz, and some generally relevant ideas from the writings of F. A. E. Pirani and S. Weinberg. But the central exercise of the work seems not to have been attempted before: it is to study the consequences of as nearly as possible applying a Lorentz transformation to the Schwarzschild exterior solution, seeking

Laser tuning

from John Walker

MOST lasers have the disadvantage of operating at only a few fixed wavelengths. Dye lasers on the other hand can now be tuned over the whole visible spectrum by selecting appropriate dyes. Fine tuning is accomplished by a variety of techniques, one of which involves birefringent crystals. A recent paper (Tang and Telle, *Applied Physics Letters*, **24**, 85; 1974) reports the observation of very large tuning rates using birefringence in ammonium dihydrogen phosphate (ADP).

Earlier work needed high gain pulsed dye lasers and used rather complicated tuning elements including intracavity polarisers and gratings. Tang and Telle employed a less powerful CW dye laser, and only a single z-cut electro-optic crystal of ADP. When the z axis was parallel to the direction of propagation of the light a voltage applied across the x direction had no effect on the laser wavelength, as symmetry considerations would predict. But if the crystal were misaligned by only one degree the wavelength then unexpectedly became a very sensitive function of voltage. Tuning rates of up to $60 \text{ \AA} \text{ kV}^{-1}$ were achieved, and they could be varied by changing the misalignment angle. Coarse tuning could be effected by rotating the ADP crystal. The precise explanation of the tuning effect is not known, but it seems to depend on having a crystal with very low residual birefringence in the absence of an electric field.

In any case, the method seems to be simple and useful, as Tang and Telle demonstrate in a second paper (*J. Appl. Phys.* **45**, 4503; 1974). Here they claim the first application of a laser to modulation spectroscopy, a technique which previously needed conventional light sources and spectrometers. The principle of modulation spectroscopy is quite simple. The spectrometer (or in this case laser) wavelength is caused to oscillate rapidly over a range of a few Ångströms. Using suitable electronics a signal corresponding to the first derivative of the absorption spectrum can then be extracted from the transmitted light. In practice this means that relatively weak spectral features are amplified and made visible. Tang and Telle studied the room temperature absorption spectrum of Nd^{3+} ions in a YAG crystal. They were able to resolve features which are normally seen only at liquid helium temperatures, and also detected some weak lines which are not usually seen at all.

The claimed advantages of the laser system over conventional modulation spectrometers are greater sensitivity because of the intensity of the laser light, and much better resolution. As a straightforward tunable laser the line width can be reduced to less than a tenth of an Ångström. The voltage tunability ensures very fine and reproducible wavelength selection. This versatile tuning system will undoubtedly find many applications.

thuswise to obtain a metric that might be interpreted as that of a mass in free (unaccelerated) motion. The procedure is to express the Schwarzschild solution in terms of the usual time coordinate and Cartesian-like spatial coordinates, and simply to apply a Lorentz transformation to these. Since the space-time tends to that of special relativity at large distances from the mass, the transformation has asymptotically its usual significance. The spatial coordinate system is not unique, but Salzman seeks to treat the passage from one to another admissible system as being analogous to a gauge transformation in electromagnetism. The two cases he treats in detail are "Schwarzschild coordinates" and "isotropic coordinates".

Except asymptotically, the transformation has none of the precise significance of the Lorentz transformation in special relativity. Of course, it may be qualitatively suggestive. One feature Salzman discusses that at first sight offers intri-

guing possibilities is a generalised Schwarzschild surface defined by the vanishing of the leading component (coefficient of the dr^2 term) of the metric tensor. In the coordinates used, however, this tensor is not in diagonal form, and so the vanishing of this component does not in general denote a singularity and therefore need not have any particular physical significance.

As regards the behaviour of a test particle in the field of a freely moving Schwarzschild mass, all the physical properties are derivable using any admissible coordinate system whatever. Since one is interested in only the relative motion of the particle and the mass, by far the most convenient system is the original Schwarzschild one where the metric takes the familiar form:

$$ds^2 = (1 - 2M/r) dt^2 - (1 - 2M/r)^{-1} dr^2 - r^2 d\Theta^2 - r^2 \sin^2 \Theta d\Phi^2$$

with $c = G = 1$.

One relevant matter is the case of

a test particle of proper mass m_0 moving radially inwards. It is easy to show that the local velocity v along the trajectory is related to the velocity V at infinity by $(1-v^2)/(1-V^2) = (1-2M/r)$. The local rate of change of momentum is then found to be $m_0 M(1-v^2)^{-1/2} r^{-2} (1-2M/r)^{-1/2}$ which may be interpreted as the gravitational attraction. This agrees with the classical value in the appropriate limit. One may interpret $M(1-v^2)^{-1/2}$ as the relative mass of the body as seen by the infalling test particle. The fact that this plays the part of the gravitational mass is one of the results got in a different way by Salzman. In most applications $2M/r \ll 1$, and we may think of r as nearly enough the classical distance. But the presence of the factor $(1-2M/r)^{-1/2}$ does recall the role of the Schwarzschild surface. So as far as this simple case is concerned, it checks that there is no change in the absolute position of this surface as it affects a moving test particle. Naturally, an observer on that particle may describe the surface in various ways depending upon what observations he makes of events in its vicinity.

The simple case might suggest that there is never any need to treat a Schwarzschild mass save with the aid of a standard description of the metric. But this is obviously not the case if we have to treat the interaction of two such masses. And this is where Salzman's work may provide a valuable start. In the first place, it supplies at any rate an approximation to the required field. In the second place, Salzman does indeed obtain by formulating a suitable Lagrangian an approximation to the behaviour of interacting masses. This Lagrangian is closely similar to one formulated by Landau and Lifshitz, which may be taken to confirm the usefulness of Salzman's metric. Incidentally, he concludes that "two-body effects can be important even in the case of a 'test' mass". Also he compares his Lagrangian with that for an analogous electromagnetic problem and pursues somewhat his analogy of gauge functions, but here the physical significance is scarcely clear. At any rate, Salzman has opened up a field in classical general relativity that will undoubtedly be further explored.

Puberty

from our *Steroid Biochemistry* Correspondent

THE mechanisms responsible for the increased secretion of androgens at the time of puberty are incompletely understood. The use of techniques for taking frequent samples of blood, the development of simple, sensitive and specific assay methods and the polygraphic recording of the stage of sleep has made possible an investigation of hormone secretion throughout a

24 h sleep-wake cycle. These techniques have shown that some hormones, for example growth hormone, luteinising hormone (LH) and follicle stimulating hormone (FSH), are secreted episodically and that their secretion may sometimes be related to the onset of a specific stage of sleep, particularly in the pubertal state. An increase of LH secretion, which may be important in initiating androgen secretion and puberty, occurring synchronously with onset of sleep was demonstrated in pubertal, and sometimes in later pre-pubertal, boys.

In a recent study (Boyar *et al.*, *J clin Invest.*, **54**, 609-618, 1974) plasma LH and testosterone concentrations were measured at 20 minute intervals throughout a 24 h period in six normal pubertal boys. In all six boys there was an increase in LH concentrations with the onset of stage 4 sleep. LH was secreted episodically and although plasma testosterone concentrations did not show such marked variations, every major secretory episode of LH was followed about 20 minutes later by an increase in the plasma testosterone concentrations. Major secretory episodes of LH could occur at times when plasma testosterone concentration was increasing and close to the maximum concentration reached during the 24 hour period, whereas during the day, although testosterone concentrations decreased, no further major episodes of LH secretions were detected. In mid-puberty similar but smaller changes were observed.

To show that the increased androgen secretion was dependent upon sleep-associated secretion of LH, subjects were studied in which sustained sleep was delayed for 3 hours. Under these conditions the first LH secretory episode again coincided with the first sustained period of stage 4 sleep and was followed by an increase in testosterone secretion. Acute sleep-wake reversal studies also confirmed this relationship, concentrations of testosterone were significantly higher during the periods asleep than during the periods awake. In contrast to these results there was no consistent increase in LH secretion with the onset of sleep in sexually mature young men and testosterone secretion occurred equally throughout the 24 hour period rather than being highest during the sleep period.

The secretion of trophic hormones such as LH from the pituitary is often regarded as being controlled by a simple negative feedback mechanism, a high secretion of trophic hormone stimulates secretion of the hormone from the target gland and this increase in turn inhibits secretion of the trophic hormone. This feedback control system seems to be operating in sexually mature young men but such a control

system does not seem to explain the changes observed during sleep at puberty when secretory episodes were often maximal at times when plasma testosterone concentrations were high. These observations suggest that at puberty the central nervous system exerts a regulatory influence which is more important than the classical feedback mechanism in controlling the secretion of LH and, indirectly androgens, at puberty. The mechanisms involved in initiating hormone secretion at this time remain to be unravelled.

Binding of streptomycin to ribosomes

from a Correspondent

STREPTOMYCIN is a well-known agent for precipitating nucleic acids. In addition to this general property, the drug is also a specific inhibitor of protein synthesis, and the inhibition is associated with the smaller (30S) ribosomal sub-particle. In *Escherichia coli*, it is known that mutations in protein S12 can confer resistance to or dependence on the drug (Ozaki, Mizushima and Nomura, *Nature*, **222**, 333-339, 1969; Birge and Kurland, *Science*, **166**, 1282-1284, 1969), and that these effects can be suppressed by mutations in proteins S4 and S5 (see for review Garrett and Wittmann, *Adv Prot Chem*, **27**, 277-347, 1973). The question therefore arises as to whether streptomycin binds to the nucleic acid or protein moiety of the 30S ribosome, and this is a problem which has given rise to some controversy.

Gorini and his co-workers believe that in the ribosome streptomycin binds specifically to the 16S RNA. In their latest experiments (Garvin, Biswas and Gorini, *Proc natn Acad Sci USA*, **71**, 3814-3818, 1974) they have compared the binding of streptomycin (SM) with that of dihydro-streptomycin (H_2SM), an analogue which can be conveniently labelled with tritium. Both drugs seem to have identical binding sites on isolated 16S RNA, as demonstrated by chasing experiments, and both induced quantitatively identical 'misreading' effects, when tested with 30S particles from various different streptomycin resistant strains of *E coli*, which bind varying low amounts of streptomycin as compared to the wild type. But the binding affinity of H_2SM is lower than that of SM, since the former but not the latter could be removed from 16S RNA or 30S sub-units by simple dialysis. This effect enabled the authors to make a very interesting experiment. 30S sub-particles from wild type or streptomycin

resistant strains were exposed to H_2SM , then the drug was removed by dialysis and the sub-particle tested in the misreading system. Surprisingly, the sub-particle showed the same extent of misreading as a control sample, which had H_2SM added back during the misreading test. Thus, streptomycin is capable of inducing a new stable conformation of the ribosome, which persists after removal of the drug.

In spite of these interesting findings, the evidence that the SM or H_2SM bind specifically to the RNA moiety of the 30S particle is still not very convincing. SM binds to 16S RNA and 30S sub-particles equally well (two moles per particle), but in an earlier publication (Biswas and Gorini, *Proc natn Acad Sci U S A*, **69**, 2141-2144, 1972) a significant binding to 50S particles and 23S RNA was also found under these conditions (0.7 and 1.3 moles respectively). Further, since the binding was measured after exhaustive dialysis rather than under saturating conditions, the number of moles bound are not necessarily saturation values. Garvin *et al* showed that SM prebound to 16S RNA could prevent the assembly of proteins under reconstitution conditions when proteins from *E. coli* B were used, whereas it had no effect when proteins from *E. coli* K were used. In the latter case, the SM remaining bound was reduced from 2 moles to 0.6 to 0.7 moles. It cannot be excluded (as the authors themselves suggest in a slightly different context) that this is due to a specific protein-mediated dislodgement of the SM from the RNA followed by rebinding to the 30S particle.

The case for binding of streptomycin to the protein moiety of the 30S particle has been put by Schreiner and Nierhaus (*J molec Biol*, **81**, 71-82, 1973). These authors distinguished two types of H_2SM binding: "Type A" (high affinity) was exhibited by 30S particles, and "Type B" (low affinity) by 50S sub-particles, and 16S or 23S RNA. Type B binding could be suppressed by high concentrations of ammonium chloride, whereas the Type A binding was unaffected, and was always considerably higher than the Type B binding. A Scatchard plot revealed that at high ammonium chloride concentration the 30S particles bound 0.8 moles of H_2SM per particle (a figure which is in close agreement with the residual binding mentioned above). Binding to 30S particles could be reduced to Type B levels by removing some of the proteins with high salt, and Type A binding could then be restored by adding back the proteins in the cold. Analysis of the individual proteins showed that S3 and S5 were the most effective in this respect. But although these experiments show a direct in-

volvement of these proteins in streptomycin binding to the ribosome, they cannot be taken as proof that the drug binds directly to the protein moiety.

So, the question of where streptomycin binds remains unresolved. At the moment the evidence seems to favour the view that the proteins are more important than the RNA, but it may well turn out that, in the complex environment of the ribosome, both nucleotide and amino acid residues are involved.

Aquatic biological pollution in Florida

from a Correspondent

OF all the ways in which man has affected the flora and fauna of the world one of the most profound has been by introducing exotic species wherever human colonies have been established. In the last century acclimatisation societies flourished with the object of introducing "beneficial" animals to various parts of the world, often with severe consequences to the native biota. Today, introductions still continue but are more often due to accidental release than deliberate introduction. A recently published study by Courtenay, Sahlman, Miley, and Herrema (*Biol Conservation*, **6**(4), 292-302, 1974) of the exotic fishes in fresh and brackish water in Florida illustrates most graphically the extent of biological pollution in this state which now contains 38 species of exotic fishes.

Thanks to its mild climate and the supply of even-temperature water from wells, Florida is the major centre in the United States for the aquarium fish industry. Some 250 fish farms exist in the state, producing nearly 80% of the pet fish for the United States. Exotic species are often unintentionally released through unprotected effluent channels, or in times of flooding. Others are dumped into open waterways when holding pools are cleared for the reception of new stock. Courtenay and his colleagues made 62 collections of fishes in central and southern Florida between July 1970 and July 1972. Thirty-eight exotic species and several hybrids between these species were found, of these 20 species and five hybrids were found to be established as breeding populations.

Not all the established exotics came directly from fish farms: some are due to release of unwanted fishes by aquarists, research workers, and to stocking by angling interests. The spread of the pike killifish (*Belonesox belizanus*) is an example. In 1957

fifty specimens were released after a medical research project was terminated, it now occurs over some 160 square miles of Dade County, and in places makes up 20% of the fish biomass, no mean feat for a slender fish of 20 cm maximum length! Others seem to be less successful—two white piranhas (*Serrasalmus rhombeus*) were caught in an (abandoned!) swimming pool in South Miami, the survivors of several after an unusually cold winter.

The walking catfish (*Clarias batrachus*) has spread rapidly and continuously since its first escape in the mid-1960s. It is an undemanding fish, capable of withstanding almost deoxygenated conditions and moderate salinity, it is also able to tolerate dessication, as well as migrating overland during rainy periods. In the dry season catfish tend to aggregate in numbers in small ponds and kill most of the animals in the pond in a few weeks.

At least eight species of cichlid fishes have become established, while others have been found. One of them, the South American black acara (*Cichlasoma bimaculatum*) is the most widely distributed exotic fish in southern Florida, and is the only exotic in the Everglades National Park. Courtenay *et al* have found that in the Fort Lauderdale area it is the dominant fish, comprising 64% of the total fish biomass. In one canal it formed 80%, but elsewhere its contribution to the fish biomass ranged from 5 to 30%. An African cichlid, the blue tilapia (*Tilapia aurea*) is now found over the greater part of northern Florida. This cichlid was claimed by the news media to be an excellent food and game fish, and was quickly spread by fishermen to other areas from its original site of introduction dating from 1961. Unfortunately it was found to be practically valueless as a game fish, and is only now being evaluated as a food fish. It has proved to be an extremely effective competitor for the native fishes, and in many eutrophic lakes now dominates the fauna.

Courtenay and his colleagues document these and the remaining exotic species in detail, but have little to say on the effects of this biological pollution on the native fauna. Undoubtedly it has been considerable, and possibly the full effects have not yet been established. They do, however, propose some remedies to stem the flood of new exotics, most particularly in drawing attention to the state statutes which already forbid the release of non-native species. Enforcement of such statutes from the outset would have prevented much of this pollution and it is greatly to be hoped that action now can contain the problem to its present, already serious limits.

review article

Leaf protein: a beneficiary of tribulation

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In some cases, the protein content of the discarded haulm of crop plants may be as great as that of the harvested crop. After two false starts at the time of the Second World War, research on exploiting this and other sources of leaf protein may now be near to acceptance.

THERE are many diverse reasons for studying the composition of leaves and preparations made from them. The extent to which preparations are refined before study is equally diverse. Research on chemical taxonomy, or the processes involved in virus multiplication, calls for the isolation of leaf components in the greatest attainable state of purity; research on the nutritive value of fodders or green vegetables calls for no more fractionation than is involved in selective browsing, or trimming in the kitchen. Research on the use of bulk preparations of extracted leaf protein as a food for man and other non-ruminants is in an intermediate position. There is no need to separate innocuous components from leaf protein—there are indeed obvious advantages in retaining components such as β carotene—but the merits of leaf protein cannot be reliably deduced from analyses of the whole leaf because protein extraction is always incomplete and it must be assumed that there are differences between the group of proteins that remains unextracted and the group that is separated as leaf protein.

The history of research on leaf protein has been published elsewhere^{1,2} and will only be summarised here. Rouelle made it in 1773, Ereky suggested its use as food in 1925 and Slade repeated the suggestion in 1937. After the outbreak of war in 1939, several groups of scientists met to discuss how scientific knowledge and skill could best be used. The conclusions were communicated to those charged with care for our welfare but no attention was paid to the suggestion that leaf protein could help to alleviate protein shortage if there were a blockade. The mental climate changed after the 'fall of France' and support for the project began. In the second half of 1940, and in 1941, large-scale equipment of many types was examined to see whether any existing machinery was suitable for extracting juice from leaves.

All types had defects. Understandably, this work stopped with the arrival of 'Lend-Lease' food.

Liberating the juice

Tribulations at the end of the war, for example the ending of 'Lend-Lease' and the growing awareness that much of the world was chronically undernourished, reawakened an interest in leaf protein that persisted for 4 or 5 yr. Interest then waned and work was languishing until we received grants from the Rockefeller Foundation and later from the Wolfson Foundation. With these grants, and on the basis of the wartime studies of machinery, several extraction units were made. The design depended on the idea that liberating juice from leaf cells differs qualitatively from expressing it from leaf pulp. Separate units were therefore used for the two processes. We concluded that rubbing is more important than fine subdivision, that rubbing leaf against leaf is as effective as rubbing leaf against steel, that the layer of pulp from which juice is being pressed should be less than 1 cm thick, that pressure should be maintained for at least 7 s to allow juice to flow away from the pressed fibre, and that there is little advantage in applying a pressure greater than 4 kg cm⁻². Units conforming to these principles have been made that handle up to 5 ton of crop per hour. The pulpers run at 1,000–3,000 r.p.m. and so waste energy in creating wind, but the presses are efficient.

The advantages of liberating and expressing juice in one operation are obvious. At various times and places rollers and screw expellers have been used with partial success, as pure pressure extracts little protein from leaves. Rollers extract protein because there is some slip, which rubs leaf against leaf,

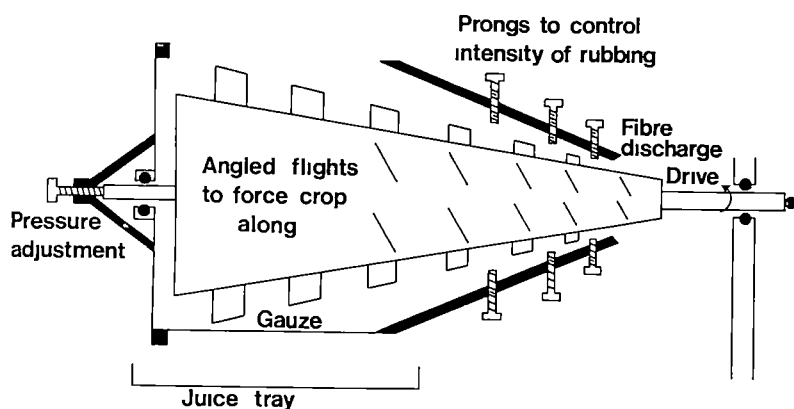


Fig. 1 Screw expeller for juice extraction

as the charge enters the 'nip', but pressure is too brief to allow juice to run away and much of it is readsorbed by the fibre. The scroll of a screw expeller rubs across the charge, this liberates juice but wastes energy in useless friction. A remodelled screw expeller in which the scroll is broken into many paddles, so that the charge is constantly rearranged and stirred as well as being pressed, should use more of the input energy to rub and tear, rather than heat, the crop. Figure 1 illustrates the principle of what is proposed. This arrangement has the additional advantage that juice is not expressed under pressure but flows by gravity through a gauze. When juice is forced through a packed mass of leaf fibre, much of the protein in organelles and their fragments is retained, and some of the finer fibre particles contaminate the juice. For quantitative agronomic studies on the yields of leaf protein attainable from different crops and systems of husbandry it will probably be better to pulp and press in separate units because it is then possible to standardise conditions more completely².

Food or fodder

Research on large-scale production of leaf protein started as a means for making human food for use in Britain and it was restarted after the war, partly for the same reason but more because it seemed likely that in the wet tropics, the yield of leaf protein would be greater than the yield of other protein concentrates. At that time the need for protein concentrates was universally agreed and the Food and Agriculture Organisation took an active interest in concentrates from such sources as oil-seeds and fish. FAO was unsympathetic towards work on leaf protein and in several publications asserted that it was unpalatable and much too expensive. Those having practical experience with properly made leaf protein knew that it was palatable—especially in countries where curry or powdered dried leaves are traditionally used as relishes. Calculations based on experience in the grass drying industry suggested that leaf protein would be as cheap as any other concentrate that was not a by-product. With FAO we were presumably up against the fundamental objections succinctly stated by Professor Mary Douglas³ "There wouldn't be time", "We couldn't afford it", "God doesn't like that sort of thing" and the ultimate "It's against Nature". The International Biological Programme was more open-minded. Research on leaf protein formed part of the national programmes of India, New Zealand, Nigeria, Sweden and the United Kingdom, and under its auspices much valuable research has been done.

Although agronomic information, and information about the effect of processing conditions on the properties of leaf protein was accumulated, there was little commercial interest. Several patents have been taken out, many of them restate facts familiar since 1773 and none cover points that are essential for commercial production. There is, or has been, commercial interest in Brazil, Eire, France, Hungary, Japan, New Zealand, Sweden, UK and USA. The actual present status of these projects is uncertain, and some of them may have failed because of the use of unsuitable equipment.

When large-scale research started it was obvious⁴ that the fibre from which juice had been expressed could be dried more economically than the forage from which it had been made. To get a ton of dry matter from a forage, 5 to 10 ton of water must be evaporated, whereas it is only necessary to evaporate 2 or 3 ton from the fibre. The product contains less nitrogen than the corresponding dried fodder but it is less lignified than a fodder with the same nitrogen content because it would have been made from a less mature crop. Furthermore, because soluble materials are largely removed from the fibre, species of leaf could be used as fodders which in the original state, are unpalatable, or even toxic to ruminants. These points were made in a note circulated by the Agricultural Research Council in 1951 and they have been stressed repeatedly (see, for example,

ref 5) since then. Even if a crop is being ensiled rather than dried extraction of juice may be prudent because it is all extracted at once, and can therefore be collected and used, instead of seeping out slowly as a polluting effluent. These advantages of fodder fractionation received little attention until the tribulations resulting from the increased price of oil focused attention on wasteful processes in general. Now, most interest is being shown in fodder fractionation as a means for conserving fodder economically, leaf protein is regarded as a by-product for feeding to pigs and poultry. There is active research at the National Institute for Research in Dairying (Shinfield), the Rowett Research Institute (Aberdeen), and the Agricultural Institute (Dublin). In some ways it is a pity that the objective has changed in this way, but the research that is being done suggests that FAO was wrong in arguing that leaf protein would be very expensive, and the experience gained will in time be used in the production of human food. Unfortunately, leaf protein will by that time have gained the stigma of being fodder and this will slightly impede acceptance by people.

Sources of leaf

Conventional crops, harvested at an immature stage, are the sources of all bulk preparations of leaf protein. At Rothamsted we prefer the cereals and clovers, while in New Zealand and USA lucerne is preferred. Batches of protein weighing a few kg have been made in Britain, India and Sweden from 20 or 30 other species—many of them normally classed as weeds. Even if species conventionally grown as sources of seed are used, it may well be that the varieties rejected by plant breeders would be better for our purpose than those that give a good yield of seed. At Rothamsted we can get 2 ton of dry extracted protein (calculated at 16% N) annually per hectare and expect to be able to get 3 ton. In India the yield is already 3 ton and it should be possible to get 5 ton. It is not certain that such large yields should be aimed at as they call for a level of fertiliser use that may not be economically sensible.

Leaves that are a by-product from a conventional crop are the ideal source of leaf protein. Potato haulm has been examined in some detail at Rothamsted, the yield of extractable protein would probably be about 600 kg ha⁻¹ at the end of July but only 100 kg ha⁻¹ in mid-September. Prudent farmers destroy the haulm at the beginning of September as a safeguard against blight, prudence would probably be more widespread if farmers realised that something valuable could be made from haulm. Bearing this in mind, and adding in the haulm from seed and early potatoes, about 60,000 ton of protein is now being wasted in Britain by destroying rather than harvesting haulm. A larger amount of protein is wasted in sugar beet tops but that source has been studied less systematically. With varieties used now, more protein can be extracted from the haulm of peas harvested for freezing and canning than is present in peas themselves. Outside leaves and other forms of waste from the vegetable industry are another large but uncertain source—uncertain because vegetables found to be surplus after reaching the shops have often deteriorated too much to be useful, and outside leaves are often damaged and would be troublesome to collect in the field. The potentialities of this source should be surveyed. One point about vegetable leaves must be stated clearly: there is no advantage in extracting protein from material that could be eaten. Few communities eat as much green-stuff as is desirable and leaf protein is more conveniently eaten in the leaf than after extraction.

Although many species now classed as weeds will be grown deliberately as sources of leaf protein, the mixed unfertilised vegetation on waste land is not a probable source. If a harvesting implement could be used on land, the land should be re-sown and properly fertilised. Water weeds are different. They are usually well fertilised and the growth in an area tends to be dominated by a single species. According to some estimates,

the annual cost of trying to control water weeds with herbicides or by mechanical destruction is £500 million hardly anything is being spent on trying to use them Enough has, however, been done to show² that leaf protein can be extracted satisfactorily from some species

Protein quality

Chloroplasts, their fragments, and other organelles begin to coagulate when leaf juice is heated to about 50° C, coagulation is complete at 70° C By separating a low-temperature coagulum, and then heating the fluid further, a green fraction containing 7–9% N and a fawn fraction containing 9–12% N can be made from most species that extract satisfactorily This may sometimes be a useful procedure, but it introduces complexities into what is otherwise an extremely simple process, the low-temperature coagulum is too soft to filter off easily When juice is heated suddenly to 70° C a dense, easily filtered curd is formed Sudden heating inactivates chlorophyllase⁶ and so prevents the formation of phaeophorbide, this was formed to a sufficient extent in slowly heated juice to photosensitise animals fed on the product⁷ The press-cake, after washing to remove soluble leaf components, should have the composition shown in Table 1 The fats are highly unsaturated This causes trouble during preservation and storage because unsaturated fat combines with

Table 1 Composition of leaf protein as usually prepared

True protein	60–70%
Lipid	20–30%
Starch	5–10%
β Carotene	1–2 mg per g
Fibre	<2%
Water soluble compounds	<1%
Ash	<3%
Acid insoluble ash	<1%

protein in a manner that makes it less digestible and keeps some of the constituent amino acids from being effectively metabolised These fats also undergo 'oxidative rancidity' This enhances the value of products such as tea and smoked fish but it is usually regarded as detrimental in other foods Dry leaf protein that has been stored in air, slowly develops a characteristic flavour As with tea and smoked fish, one learns to like it but, in the early phases of popularisation, the less flavour it has the better

Protein does not extract well from leaves rich in tannin or phenolic compounds Even small amounts of these substances, though they may not prevent extraction, combine with the protein as unsaturated fats do Differences in the extent to which these complexes were formed during extraction or storage, probably explain the differences in nutritional value that have sometimes been observed between samples of protein with similar amino acid compositions It will probably not be possible, in the course of bulk production, to prevent combination between protein and phenolic compounds That form of damage could be circumvented by selecting species and stages of growth that ensure that the leaves are relatively free from phenols

Some people dislike the green colour of leaf protein made by the simple method outlined Extracting the colour with a solvent is easy—but it adds to the cost, and the useful β carotene and unsaturated fats would then be lost Furthermore, leaf protein production has not only the merit of yielding an unprecedentedly large amount of edible protein, it is also so simple that it could be a small-scale industry in any community able to manage a tractor It will lose that merit if solvent extraction is insisted on

All leaf protein preparations for which there are reliable figures have similar amino acid compositions⁸ It is unlikely that

species differences are greater than differences between preparations made from the same species harvested at different ages or in different conditions of husbandry This uniformity is not unexpected because what is loosely called leaf protein is a mixture of many different proteins and the same amino acid deficit or excess is not likely to characterise all of them Amino acid analysis suggests that properly made leaf protein will have good nutritional value This expectation was borne out by experiments on the growth rate of chickens, pigs and rats and by brief experiments on the amount of nitrogen retained by infants fed on milk alone and on mixtures of milk and leaf protein These results have been confirmed² and extended by a six month experiment in which school children, on a diet consisting predominantly of ragi (*Eleusine coracana*), were given supplements containing 10 g of protein per day in the form of lucerne leaf protein, or sesame seed (*Sesamum indicum*) The leaf protein gave better results, assessed from height, weight and haemoglobin content, than sesame although the protein in the latter is regarded as one of the best seed proteins

Acceptability

Leaf protein should not be regarded as a medicine and eaten in the form of pills It should rather become one of the many components from which a mixed diet is made up But people do not, as a rule, think of their diets in terms of so much energy, so much protein and so on per day They eat foods with familiar appearance for traditional reasons Methods must, therefore, be found for presenting any novel foodstuff in an acceptable way Fresh leaf protein is a slightly acid cake containing 60% water and with the texture of cheese It can be pickled, salted or dried It will be most readily accepted by communities that already make regular use of green vegetables and, as already mentioned, increased use of leafy vegetables is the most sensible way of getting leaf protein eaten The next step depends on local culinary habits As a result of present-day cultural interpenetration, the food shown in advertisements in publications coming from wealthy countries are gaining prestige, while the traditional stews and gruels of much of the world, into which many novel foods, such as leaf protein, would fit easily, are losing it This is understandable but unfortunate because the foods of the wealthy are getting scarce so that expectations that are not likely to be fulfilled are being fostered It is likely therefore that novel foods, on which work was originally undertaken so that they could relieve malnutrition in developing countries, will not be acceptable there until they are manifestly used by the wealthy Acceptance will be impeded if they are first used as welfare foods or foods for the unfortunate

In some parts of the world the need for protein could be most economically satisfied by growing yeast or some other micro-organism on molasses or oil In others the best source may be a seed such as groundnut or soya But in rural tropical regions where rainfall is regular, or even excessive, leaves are the most productive source of protein these regions are at present the worst fed parts of the world Tribulation has thrice energised research on the production of leaf protein and on its use as food for people and other non-ruminants Research may now have progressed so far that the logical case for production of leaf protein is strong enough to prevail against habit and preconception It is possible, however, that a fourth jolt will be needed If economists and other prophets are to be believed, no scarcity of tribulation is imminent

¹ Pirie, N W, *Science*, **152**, 1701 (1966)

² Pirie, N W (ed.), 'Leaf protein: its agronomy, preparation, quality and use' IBP Handbook 20 (Blackwell, Oxford, 1971)

³ Douglas, M, in *Ecology in Theory and Practice* (edit by Benthall, J.), 129 (Viking Press, New York, 1973)

⁴ Pirie, N W, *Chemical Ind*, **61**, 45 (1942), *Nature*, **149**, 251 (1942)

⁵ Pirie, N W, *Fertil Feed Stuffs J*, **63**, 119 (1966)

⁶ Arkcoll, D B, and Holden, M, *J Sci Fd Agric*, **24**, 1217 (1973)

⁷ Lohrey, E, Tapper, B, and Hove, E L, *Br J Nutr*, **31**, 159 (1974)

⁸ Byers, M, *J Sci Fd Agric*, **22**, 242 (1971)

articles

Earthquake simulation by nuclear explosions

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A country wishing to evade a comprehensive test ban treaty could disguise a clandestine nuclear test as a natural earthquake by detonating a sequence of explosions, one of which would be caused by the device of interest

ANY discussion of a treaty prohibiting underground nuclear weapon tests (that is, a comprehensive test ban treaty, CTBT) must address the crucial issue of how to monitor whether or not the treaty is being observed. Much of the current literature ignores the possibility of schemes to conceal or disguise clandestine testing, so here we describe the reasons for believing that illicit nuclear tests, with yields up to at least 100 kilotons (kton) are feasible. Further, such tests can be carried out with little likelihood of discovery.

Seismic monitoring

The seismological literature describes numerous techniques that are directed towards identifying the source of a seismic signal as either an explosion or an earthquake. In general, they are based on features of the signals that can be explained in terms of the known gross differences in physical source mechanisms between earthquakes and single explosions. Elements of confusion enter into the use of all known discriminants, however, because the real Earth is far from an idealised, homogeneous body. Interfaces within the Earth, for instance, convert some of the compressive (P) wave energy into shear (S) waves, this tending to remove a deficiency that would otherwise be a tell-tale characteristic of explosion signals. Rayleigh and Love waves transmitted along the Earth's surface are nonetheless generally stronger in the radiation from an earthquake than in that from an explosion, but the quantitative differences are not dependable, and even though the first motion of the recorded signal from the two source types would, in principle, be distinctly different, natural background noise in general obscures it.

The salient differences between earthquake and explosion are:

- The first motion of earthquake seismic waves is rarefactional in some directions (sometimes only along rays confined to 100–200 km from the source). Seismic-wave first motions from an explosion, on the other hand, are everywhere compressional.
- P-wave trains from an earthquake are often extended in time whereas those from an explosion tend to be more compact. This results in greater P-wave 'complexity' for some earthquakes than for explosions¹.
- Seismograms from deep earthquakes frequently exhibit a phase (the pP phase) caused by reflection from the Earth's surface above the earthquake. This follows the initial downward-directed P phase by a time interval proportional to the focal depth. This interval also increases with the distance to

the detection station, an effect called 'stepout'. Explosions are seldom deep enough to exhibit a clearly separated pP phase or stepout.

- The surface waves generated by an earthquake carry relatively more energy than do those generated by an explosion of the same body-wave magnitude. Thus the surface-wave magnitude, M_s , for an earthquake is usually greater than that associated with an explosion of the same body-wave magnitude, m_b (ref 2).

Seismic evasion

We have attempted to simulate the seismic signal of an earthquake by superposing eight seismograms of a single explosion. These were identical in shape, being simply scaled versions of the explosion seismogram actually recorded at a single monitoring station. Each seismogram was delayed in time relative to the first and scaled in amplitude to correspond to that of an explosion of selected yield. This procedure was carried out for each of three different monitoring stations.

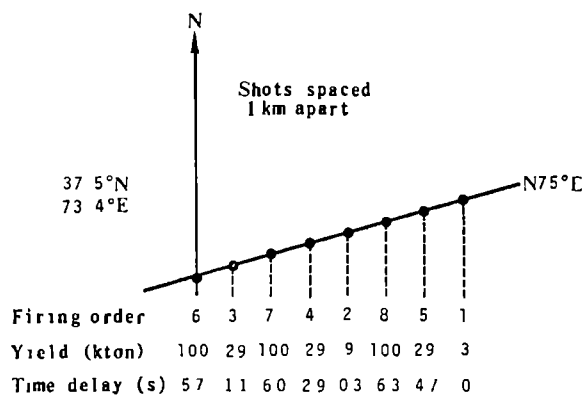


Fig 1 Geometry, firing order, yields and time delays of the eight explosions selected to simulate an earthquake

Figure 1 shows the proposed spatial and temporal separations of the shot array. In order to take travel times realistically into account we postulated a specific test site, at 37°5'N and 73°4'E in southern Tadzhik, SSR, coinciding with the epicentre of the earthquake we used for comparison. (Lack of a suitable comparison earthquake record prevented us from choosing a more realistic site, such as the area around Lake Baikal.) The azimuth, N75°E, was chosen arbitrarily and because detonation under a river would present many advantages, such as masking surface disturbances both before and after detonation, we chose a linear array of explosions.

We formed the composite, synthetic seismograms at the

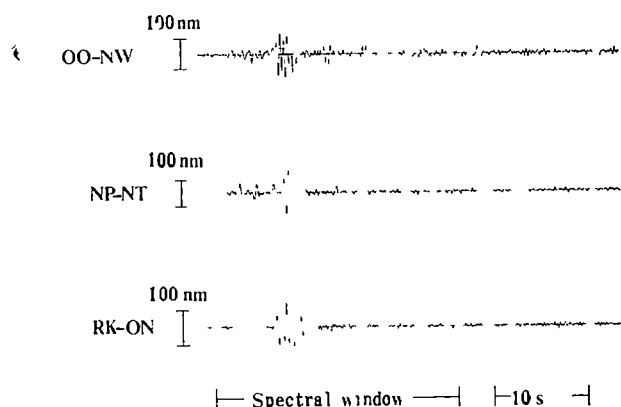


Fig 2 Composite, short-period, synthetic seismograms for the shot array of Fig 1 as they would be recorded at three seismic stations

three seismic stations Mould Bay, Northwest Territory (NP-NT), Oslo, Norway (OO-NW), and Red Lake, Ontario (RK-ON). The operations involved were

$$P(t) = \sum_{i=1}^8 p(W_i, t - t_i - T_i) + n(t)$$

$$p(W_i, t) = (W_i/100)^0 \cdot p(t)$$

where $P(t)$ is the composite, synthetic seismogram at a station and $p(t)$ is the filtered seismogram of a Semipalatinsk (Soviet nuclear test site) event as recorded at that same station. The other quantities appearing in the equations are $n(t)$, a micro-seismic noise recording, T_i , the individual travel times from explosion point to detector site, and W_i and t_i , the yields and delay times specified in Fig 1.

The United States Coast and Geodetic Survey (USCGS) reported that the explosion which generated $p(t)$ occurred on March 3, 1965 at 06 14 57 GMT. The event coordinates are 49° 8' N, 78° 1' E with depth constrained to the surface of the Earth. From the body-wave magnitude, $m_b 5.6$, we infer a yield of about 100 kton in hard rock.

The sequence in Fig 1 was not optimised for evasion. It is presented only as an example of a sequence whose seismic record would probably be identified as that of an earthquake.

Figure 2 shows the composite, short period, synthetic seismograms for the three stations and Fig 3 the seismograms of an earthquake that occurred at the postulated test site on February 2, 1965. The USCGS reported the earthquake origin time as 15 56 51.0 GMT and the magnitude as $m_b 5.8$. The two sets of seismograms are sufficiently similar that such qualitative inspection alone is unlikely to arouse an analyst's suspicions.

The following observations are based on more detailed examination of the synthetic records.

- First motions might be interpreted as refractional in two (OO-NW and NP-NT) of the three examples presented. Identification as an earthquake on this basis is unlikely, however, as the low signal-to-noise ratio makes the interpretation ambiguous.

- The three high yield explosions produce signals very suggestive of a pP phase. Again, the uncertainties are such that this could not be regarded as identifying the event positively as an earthquake. This aspect is not crucial, however, for many seismic regions that are suitable as testing sites produce earthquake signals lacking such a reflection phase.

- The body-wave magnitude would be about 5.1, equivalent to the magnitude of a 50-kton explosion in hard rock.

- The time duration of the composite P-wave signal is longer than that appropriate to a 100-kton explosion and its complexity is greater.

- We examined the Fourier spectra of the explosion on March 3, 1965 and the earthquake on February 2, 1965 at each of the three stations. The shot array spectra show no systematic pattern of peaks and nulls suggestive of source interference phenomena and overall comparison of the two sets of spectra does not reveal any other features indicative of the shot sequence.

- Rayleigh waves detected at long ranges, whether from an earthquake or an explosion, have periods chiefly in the 20-s band. Thus, the separate signals from a series of explosions occurring within a few seconds of one another will superpose constructively.

We illustrated the latter point by superposing (with itself) the Rayleigh wave signal from an earthquake which occurred in the Kermadec Islands on March 3, 1965 (The USCGS origin time and magnitude were 05 52 57.4 GMT and $m_b 4.7$, respectively). This signal was scaled, shifted and superposed in the same way as the short period signal. The amplitude of the resultant superposition is about three times larger than that of the largest individual signal. Accordingly, the surface-wave magnitude M_s of the superposition is about 0.5 units greater than that expected from a single 100-kton explosion. We used the Rayleigh waves from an earthquake because the Rayleigh waves from the explosion on March 3, 1965 were obscured by the seismic signals from the earthquake at two of the three stations and were too weak to observe at the third. This substitution does not however, affect our argument. Rayleigh waves from earthquakes have the same periods as those from explosions and we are showing only the effects of superposition.

The net effects of modifications to body and surface-wave magnitude are shown in the M_s , m_b plot, characteristic of the postulated shot array region (Fig 4, adapted from Fig 7 of ref 3). The signal from a single 100-kton explosion would be expected to lie within box 1 (the width of which is intended to be representative of the spread in M_s values associated with a given m_b). The body-wave magnitude of the superposition is reduced to 5.1 which would result in an M_s , m_b value lying within box 2 provided the surface wave magnitude were that corresponding to a single 100-kton explosion. Enhancing M_s by 0.5 units (the effect of constructive interference of the surface waves) moves the point into box 3. This falls comfortably within the earthquake population.

Thus the signal, when subjected to typical discrimination analysis, appears more earthquake-like than explosion-like.

Practical considerations

We have studied the engineering, logistic, camouflage and diagnostic considerations that would be crucial to the actual conduct of such a clandestine test. Although a detailed discussion

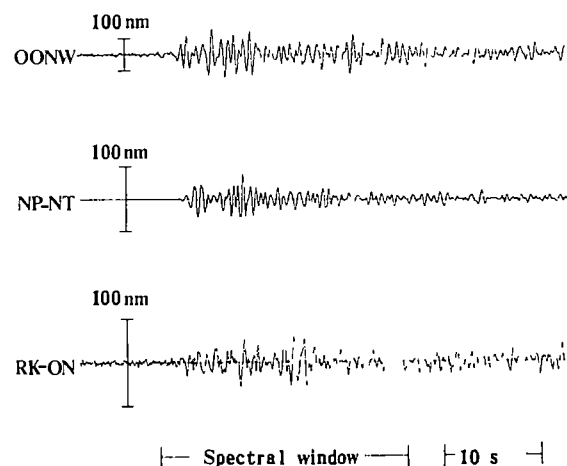


Fig 3 Seismograms showing P waves of an earthquake near the Russia-Afghanistan-China border intersection as recorded at three seismic stations

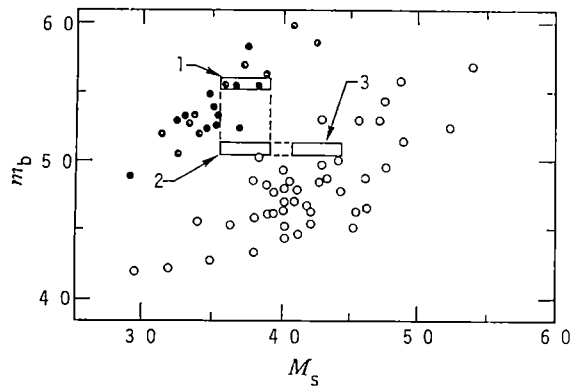


Fig 4 Surface-wave magnitude (M_s) against body-wave magnitude (m_b) for a population of earthquakes and explosions and for the eight-shot array discussed here. All events are from a region around the assumed test site. Adapted from ref 3. ●, Explosions, ○, earthquakes

lies outside the scope of this article, we conclude that none of these matters would present serious problems.

Briefly summarising, camouflage might well be accomplished by detonating the explosives under a river bed. We have carried out a complete feasibility study of this unconventional operation. Including the conceptual design of a suitable drill barge and its associated drilling equipment, the development of complete pre-shot and post-shot operational plans, the development of a drilling plan, including operation of a drill-barge, design and emplacement of a canister containing both device and diagnostics and device detonation. The important result was that the whole operation could be accomplished with current technology, albeit assuming the high level of technical competence in the domain of nuclear testing to be expected of mature nuclear powers.

The operational concept was shown to evade all possible detection threats, for example

- Increased river activity is accounted for to the local populace by initiation of a suitable construction project
- Overhead surveillance is thwarted by carrying out drilling or other identifiable operations only at night
- Drilling chips are discharged downstream, in deep water
- Surface expressions are averted by detonating the explosions underwater

We acknowledge that such an operation would call for special care and effort and would entail greater expense than non-prohibited tests. In the feasibility study alluded to we also considered these matters. We are confident that the additional difficulties resulting from the clandestine nature of the test can be overcome at acceptable levels of expense and effort. With dedication and suitable inducement a test crew of reasonable size (~100) could carry out the entire operation in about four months. This conclusion is again based on US test experience and short extrapolations from that. We estimated that costs would not exceed those of a single conventional test by more than a factor of 30. Thus, although such a test would be expensive, its cost need not deter a determined evader.

If, on the other hand, the drilling were done on land, avoidance of the visible or other detectable effects of a clandestine explosion would become a prime requirement. Potential effects include, primarily, various alterations of ground surface contours over the explosion and the release of radioactive effluents. A careful evader with test experience could predict such effects with confidence.

Surface collapse craters are common and very visible phenomena at underground nuclear explosion sites⁴. Their probability of occurrence depends on the local rock type as well as the yield and depth of the explosion. Experience in the United States shows that a burial depth (in metres) of at least 300 times the cube root of the yield (W), expressed in kton, will prevent surface cratering regardless of the rock type.

Other visible effects can include minor surface cracking related to ground disturbances and settlement, and surface expression of fault motion stimulated by the explosion. These are observed within a few kilometres of US tests, where the typical burial depth is in the range of $120W^{1/3}$ (ref 5). Offsets of a few centimetres are common and cases of dislocations measuring a metre or more have occurred. Burial at greater than normal depth would probably, though not certainly, eliminate these effects. Gasbuggy, Rulison, and Rio Blanco, the three deep US nuclear explosions fired to test stimulation of natural gas production, produced no observed fault motion or surface cracking. Their depths of burial were all at least $400W^{1/3}$ m.

Venting of radioactive material would in all likelihood be completely absent and at most would be limited to trace seepage of gases, detectable only by sensitive survey instruments at the explosion site. Present containment techniques in use by the United States are highly successful at normal burial depths and would unquestionably assure containment at the greater depths appropriate to a clandestine explosion.

Finally, and very briefly, precautions of a practical nature which an evader would likely take are

- Carry out the test in an area of low population density with at least moderate seismicity
- Use the smallest possible test crew
- Devise a cover operation to deceive the local populace and provide an excuse for whatever relocations might be necessary
- Detonate before dawn to catch most of the local population asleep. Avoid detonation on an even hour, half hour or minute, as has been common practice in previous non-prohibited tests
- Issue a routine earthquake announcement. The details would be misleading (but not so misleading as to arouse suspicion)
- Select highly reliable explosives to be included with the test device in the explosion sequence (We did, however, study the effect of failure of any one of the eight shots, but it is small and does not change the conclusions presented here.)

We believe that these precautions, and others, can be followed by a determined, resourceful evader with previous field test experience and a stockpile of proven, reliable weapons.

Reassessment

We have outlined in this article a possible way of carrying out clandestine nuclear tests. The arrangement produces a seismic signal that would be identified by seismic analysts as that of an earthquake on the basis of the M_s , m_b criterion. All other criteria considered are at least consistent with, or suggestive of, identification of the event as an earthquake.

We made no attempt to optimise yield, number of explosions or any other parameter, or to identify the maximum yield that could be tested. Even so, we demonstrate a substantial enhancement of yield capability over the detection threshold for an undisguised test.

Further, the maximum yield permitted by this evasion scheme would still not tell the whole story. Weapons of still higher operational yield could be developed and stockpiled if an evader were willing to risk extrapolating their expected performance from partial-yield experiments.

We are aware of intensive current work on new discriminants, for example, Love-to-Rayleigh spectra ratios, radiation patterns and newer techniques for depth determination. These newer discriminants are generally incompletely developed, but even if they were applied the superposition presented here would be classified as an earthquake according to present standards. It met the M_s , m_b criterion and, as discriminants are used at present, satisfaction of just one discriminant results in identification (This is because any requirement that more than one criterion be satisfied would preclude identification of most events).

We believe that recent statements concerning identification of nuclear tests in the 10-kton range must be understood to

apply only to the current open mode of underground testing. If a CTBT were to emerge, the relevant seismic monitoring capability should take into account competent efforts at evasion. We suggest that such a reassessment must be factored into the proposed safeguards for a CTBT.

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The subunit structure of the eukaryotic chromosome

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New structural data have been obtained from neutron scattering studies of chromatin. The concentration-dependent meridional peak at 10–11 nm comes from the interparticle spacing of a subunit structure. Peaks at 5.5 and 3.7 nm have a different contrast behaviour to those at 11.0 and 3.7 nm showing that histones and DNA have a different spatial arrangement in the subunit. A globular model in which apolar segments of histones form the core surrounded by DNA complexed with the basic segments of histones agrees with the data.

STRONG evidence for a basic structural repeat in eukaryotic chromosomes comes from the observation of a series of rings at about 11.0, 5.5, 3.7, 2.7 and 2.2 nm in the X-ray patterns of native and reconstituted chromatin^{1–8}, they do not seem to have been observed in the X-ray pattern of DNA nor in the X-ray pattern of total histone. In 1964, Wilkins proposed a model of a uniform supercoil with a pitch of 12.0 nm and an outer diameter of 13.0 nm to explain this characteristic X-ray pattern, and in the model calculations it should be noted that only the X-ray scatter of the DNA component was considered⁸. Another, more irregular supercoil model (pitch 4.5–7.0 nm, outer diameter 8.0–12.0 nm) has been proposed⁹. An extension of the idea of the supercoil is a polyhelical model¹⁰. Several authors have proposed a globular subunit structure for chromatin^{11–15}. 'Linear arrays of spherical chromatin particles have been observed'^{11–13}, of about 7.0 nm diameter joined by threads 1.5 nm diameter. This 'particles-on-a-string' model is supported by further observations: endonuclease digestion of chromatin gives DNA pieces in integral units of about 200 base pairs^{16–18}, and nuclease digestion a basic subunit of 205 base pairs and a second degradation subunit of 170 base pairs¹⁹. It has also been postulated¹⁸ that tetramers of histones (H3 and H4), observed in histone gently dissociated from chromatin^{20–21}, associate with 200 base pairs to give a subunit of chromatin.

Clearly, additional data are required and for this reason we have used neutron techniques. Small angle neutron scatter

techniques have particular application to studies of structures of complex biological systems at low resolution^{22–25}. The very large difference between the average neutron atomic scattering factors per atom for H₂O (-0.06×10^{-12} cm) and D₂O ($+0.63 \times 10^{-12}$ cm) allows the scattering from all biological molecules to be contrast-matched. Figure 1 shows that neutron scatter from DNA is expected to be matched at about 63% D₂O and that of

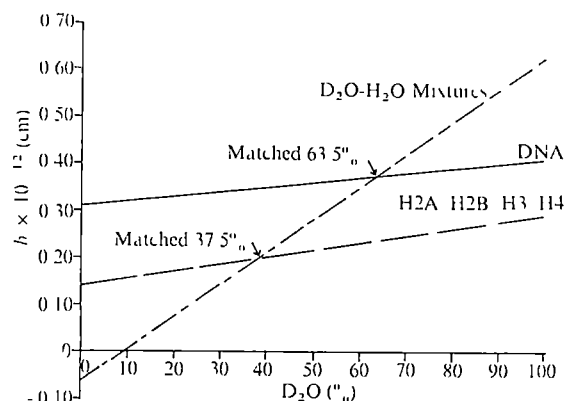


Fig 1 Computed neutron scatter from the components of chromatin. Mean neutron atomic scattering amplitude (b) against percentage D₂O in the D₂O + H₂O mixture for H2B, H2A, H4 and H3 histones together, and for DNA. The plots for histone and DNA are sloping because of the exchange of labile protons with deuterium and thus is proportional to the amount of D₂O in the aqueous mixture.

histones at about 37.5% D₂O. In practice, however, using hydrated H1-depleted histone films, the matching occurs at 44% D₂O. The large difference in the proportions of D₂O required to match histones and DNA provides an opportunity to study the relative disposition of histones and DNA in chromatin.

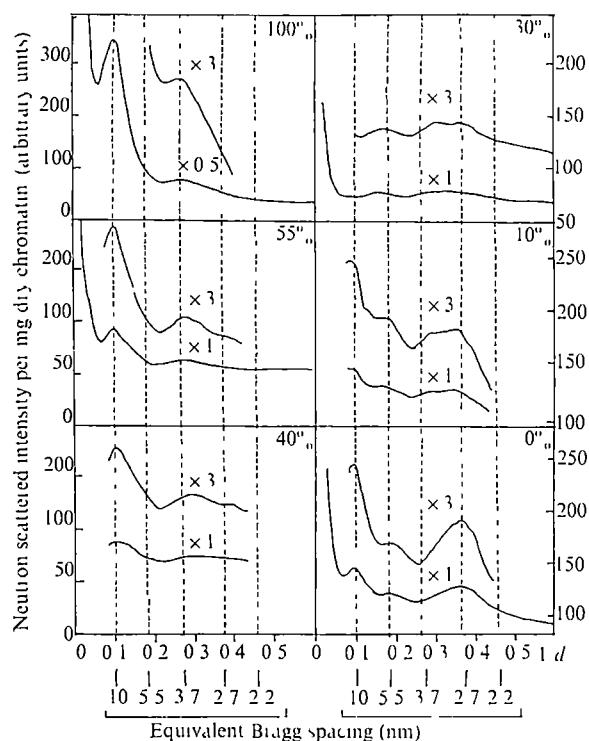


Fig. 2 Contrast variation of the radial distribution (averaged over 360°) of neutron scattered intensity for total chromatin films at 50% w/w concentration, $1/d$ is the reciprocal of the equivalent Bragg spacing (given below). The chromatin was isolated from fresh calf thymus by the method of Panyim *et al.*³⁶ Chromatin and H1-depleted chromatin³⁷ were treated with 0.5 M HCl for 2 h at 4°C , precipitated with six volumes of acetone and washed three times with acetone. After vacuum drying the histones were analysed by polyacrylamide gel electrophoresis³⁸. The protein/DNA ratios were measured by infrared spectroscopy, with suitable corrections as reported previously³⁹, which also shows any sample impurities. Experiments were carried out at 6 \AA with the small angle scattering equipment (NLS)²⁴ at the ILL, Grenoble. The graphs have been redrawn on different scales, the factors by which the originals were changed being indicated on the appropriate curve. The percentage D_2O is given in each case.

Contrast variation studies in chromatin

Contrast variation experiments, using H1-depleted and total chromatin gel equilibrated with vapour from mixtures of H_2O and D_2O , provided results over a range of relative humidities (r.h.). Those for 50% w/w concentration of chromatin at 98% r.h. are shown in Fig. 2. Although the resolution is lower than for X-rays, the curve which approximates to the X-ray patterns is that at 10% D_2O , which is also approximately the composition at which the solvent neutron scatter is almost zero. The effect of changing the $\text{H}_2\text{O}/\text{D}_2\text{O}$ ratio is striking. At 100% D_2O the 10.0 nm (approximate) ring is dominant and there is a clear ring at 3.7 nm. As the D_2O content is reduced the intensity of this 10.0 nm ring weakens to become zero at 30% D_2O , after which it increases again. A plot of the square root of the intensity is linear and goes through zero at 30% D_2O . The background scatter underlying the diffraction rings also levels at the same $\text{D}_2\text{O}/\text{H}_2\text{O}$ ratio. The 3.7 nm ring behaves similarly but retains a low intensity at 30% D_2O (Fig. 2). The 5.5 and 2.7 nm rings, however, are strongest at 0% D_2O and weaken in intensity with increasing D_2O content, until the 5.5 nm ring is no longer observable and the 2.7 nm ring is very weak. Similar results were observed with H1-depleted chromatin at about 9% w/w, except that the intensity of the lowest angle ring is reduced to zero at about 44% D_2O . At 77% w/w, the lowest angle ring is found at 9.0 nm, reaching zero intensity at 0% D_2O . The 10.0 nm ring seems to be a Bragg peak in a

region where scattering comes mainly from the protein component of the chromatin subunit. The other peaks may be part of the Fourier transform of the individual subunits, the 5.5 and 2.7 nm peaks arising from the transform of the DNA component and the 3.7 nm peak from the arrangement of proteins in the subunit. The background scatter also seems to come from the protein component.

One of the chromatin films used was stretched and the neutron scatter pattern recorded after equilibration in 98% r.h., 100% D_2O . Figure 3 shows that the 10.5 nm ring has a meridional orientation and the background scatter a strong equatorial orientation. With other camera settings, this equatorial scatter extended to about 4.0 nm equivalent spacing.

Concentration dependence in H_2O and D_2O

There is a marked concentration dependence of the intensities of the low angle X-ray rings of chromatin^{1,2,6}. With increasing concentration the ring at about 11.0 nm weakens and disappears, whereas the higher angle rings in sequence initially increase in intensity then weaken and disappear. Finally, in the dry state two rings are found at about 8.0 and 4.0 nm. Attempts have been made to explain this behaviour by changes in the packing of regular supercoils⁵. With neutrons a similar behaviour is observed for chromatin in H_2O (Fig. 4). The neutron ring at about 10.5 nm weakens with increasing concentration reaching zero intensity at 77% w/w and re-appearing at 8.0 nm at the highest concentrations. At 77% w/w, the background scatter underlying the diffraction rings also levels in a manner similar to the contrast matching against $\text{D}_2\text{O}/\text{H}_2\text{O}$ ratios described above. Again, the resolution is lower than for X-rays, the intensities in the other rings seem to move to higher angles with increasing concentration. In D_2O , there is a markedly different concentration dependence of the intensities of the diffraction rings (Fig. 5), crucial to our understanding of the chromatin diffraction patterns. In contrast to the behaviour in H_2O the lowest angle ring does not go through zero intensity with increasing concentration. Because of the similarity of H_2O and D_2O this difference, and therefore the concentration dependence of the intensities of both X-ray and neutron rings in H_2O , cannot be ascribed to structural changes. With increasing concentration, however, the peak moves from about 10.5 nm to 8.4 nm, consistent with closer packing of the chromatin subunits. Throughout the changes in D_2O , the 3.7 nm peak is constant, indicating a different origin to the variable 11.0 nm ring.

Neutron scatter from histone and DNA

We were particularly interested in the contrast variation of the low angle scattering from histone. Neutron scatter curves from films of total histone and H1-depleted histone show a clear

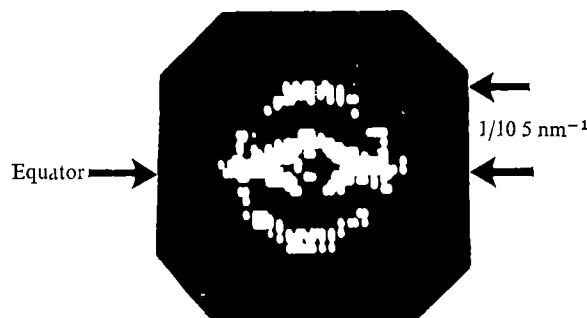


Fig. 3 Neutron scatter pattern (at 98% r.h. D_2O vapour) from an H1-depleted chromatin film which was stretched to produce orientation in the vertical direction. The pattern was reproduced on a display screen representing about 4,000 detectors of equal sensitivity over the area of the picture. The screen is 'brightened up' over a restricted range of detector counts. Wavelength, 12 \AA .

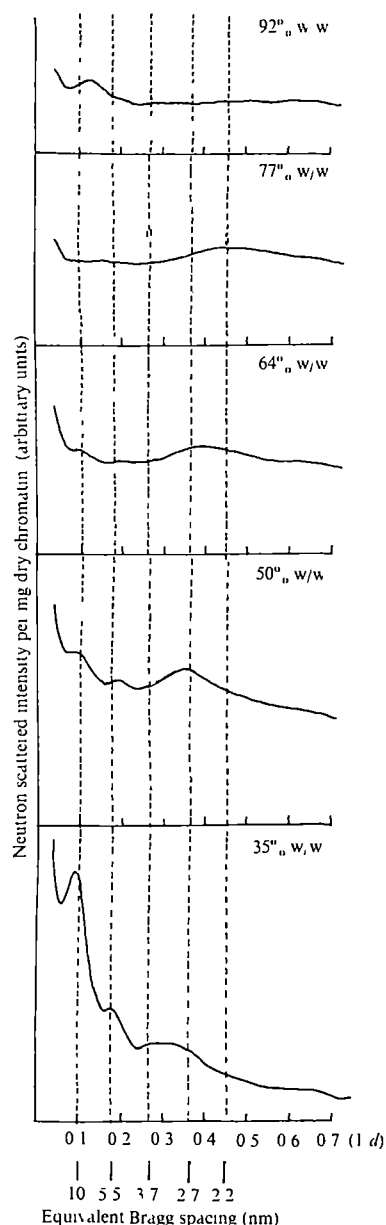


Fig. 4 Concentration dependence of the radial distribution (averaged over 360°) of neutron scattered intensity in H_2O for total chromatin. The distribution is superimposed on a background of incoherent scattering (not shown). The results for H1-depleted chromatin were very similar. The chromatin was depleted in histone H1 using an ion exchange resin⁸⁷ (Bio-Rad AG50W-X2) with a final buffer concentration of 0.65 M NaCl, 10 mM Tris-HCl, pH 7.

diffraction peak at 13.0 nm (equivalent Bragg spacing) in hydrated histone films. This ring is probably related to the scatter at 11.0 nm in native chromatin and reached zero intensity at about 44% D_2O content, a similar result to that obtained for the low angle scatter peak from H1-depleted chromatin mentioned above. We have also studied the contrast behaviour of films of DNA hydrated at 100% r.h. A strong neutron scatter peak corresponding to the packing of DNA behaved in a manner similar to that exhibited by the 2.7 nm peak, with change of D_2O content, shown in Fig. 2.

Discussion

The use of 'contrast matching' in low angle neutron scatter studies of chromatin shows that the low angle rings at about 11.0, 5.5, 3.7 and 2.7 nm originate not from the spacing of a single structural repeat and its higher orders (regular supercoil

model) but from different spatial arrangements of the histones and the DNA. The arrangement of histones, H2A, H2B, H3 and H4, provides a broad distribution of neutron scatter which is strong in the region of the concentration-dependent 10–11 nm peak and also has a strong component at 3.7 nm, whereas the DNA component contributes to the rings at 5.5 and 2.7 nm. Thus the region of neutron scatter to which the two main

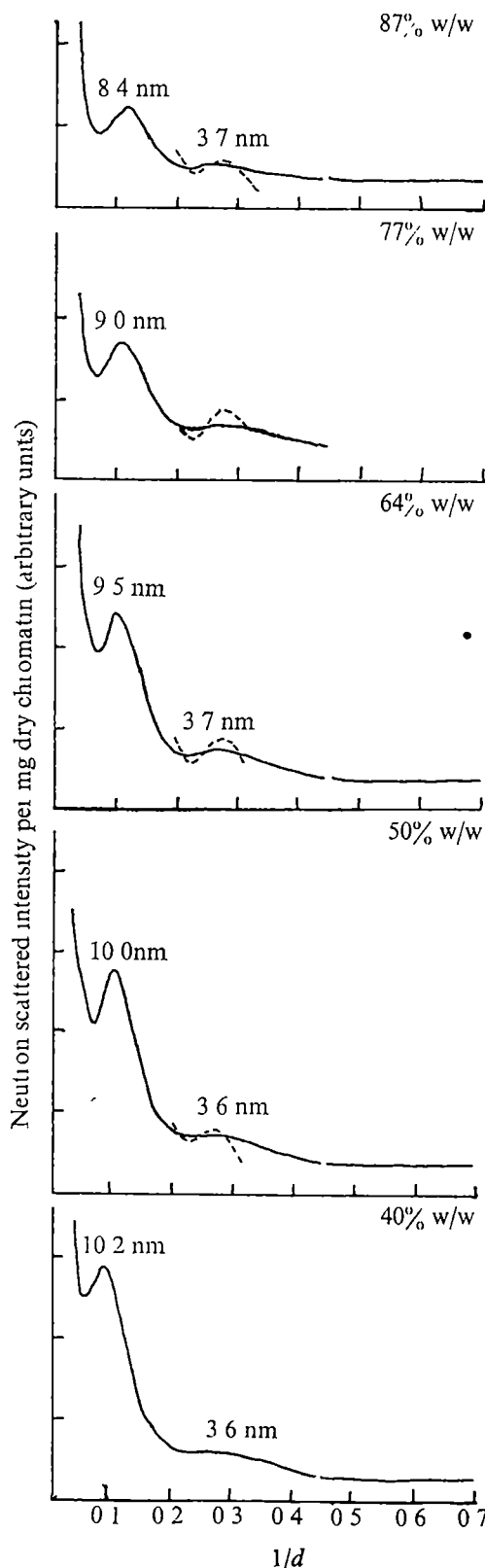


Fig. 5 Concentration dependence of the radial distribution (averaged over 360°) of neutron scattered intensity in D_2O for total chromatin. $X\%$ is the percentage concentration of dry chromatin in the films, obtained by bringing the films to a constant weight in a vacuum after the experiment. The dotted curves represent vertical magnification by a factor of 6.

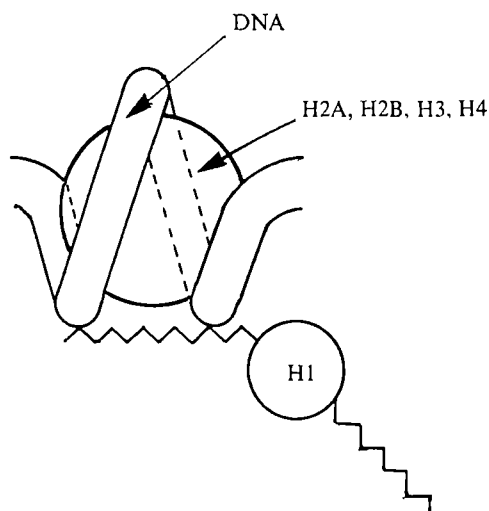


Fig 6 Schematic representation of a possible model for the chromatin subunit structure. The protein core is a complex of the apolar segment of the four histones indicated, the basic segments of the histones being complexed with DNA on the outside of the unit. Histone H1, possibly on the outside of the chain of globular subunits, may have a cross-linking role, either between subunits in the same chain or between subunits in different chains. The pitch of the DNA, which need not be uniformly coiled, is 5.5 nm with a mean diameter of about 10 nm.

components of chromatin contribute can be largely separated even though it is strictly a case of adding together all the scattering vectors of the whole structural unit to obtain intensities.

Clearly the concentration-dependent ring at about 10.5 nm arising from protein scatter can be attributed to the interparticle spacing of the proposed subunit structure for chromatin. Together with the low angle ring obtained from the neutron scatter of total and H1-depleted histone, this suggests that histones H2A, H2B, H3 and H4 comprise a multimeric protein unit, occupying a separate region of space to the DNA component. Neutron scatter data suggest that DNA is external to the histone. The equatorially oriented low angle scatter distribution (Fig 3) extends out to equivalent spacings as high as about 4.0 nm, using an analysis analogous to Clark-Jones²⁶ this may be interpreted in terms of the equatorial transform of the individual subunits in the string. The contrast matching experiments show that this scatter and the background scatter in more dilute solution comes from the low angle component of the protein transform, which is presumably at a higher angle than the low angle component of the DNA transform. This would be expected from a globular unit containing a core of histone surrounded by DNA. It is not clear whether this protein core is spherical or extended along the fibre direction, the directional properties of this structure will involve contrast and concentration studies on oriented chromatin films to seek out zeroes in the subunit Fourier transform (*c f* 40).

The difference in concentration dependence of the intensities of the low angle rings in H₂O (Fig 4) compared to D₂O (Fig 5) could be explained by the contrast matching of the protein unit against a matrix of hydrated DNA. Histones contain highly basic segments and NMR studies show that these segments (for example, 1-31 of histone H4, 1-31 of histone H2B, 1-25 of histone H2A) are the primary sites of interaction with DNA, whereas the complementary apolar segments (in which the secondary structure can be induced by salt) are the sites of histone-histone interaction^{27,28}. Similar interaction of the apolar segments would be expected in the observed interaction between different histones²⁹. The neutron data clearly show regions of protein separate from DNA, we propose

that these regions are complexes of the apolar segments of the histones H2A, H2B, H3 and H4. When the chromatin is diluted water will preferentially hydrate the DNA, in the neutron scatter, therefore, the apolar protein cores will be contrasted against a matrix of hydrated DNA. Figure 1 shows that the protein neutron scatter density lies between that for DNA and water, only in H₂O and at low D₂O contents. As the H₂O hydration of DNA changes with concentration the scatter of the hydrated DNA, at a particular state of hydration, can therefore match that of the protein. With D₂O no such concentration-dependent contrast matching would occur because the neutron scatter densities of both DNA and D₂O are greater than that of the protein. The 3.7 nm ring, attributable largely to protein scatter, does not move during hydration. This possibly arises from the arrangement of proteins in the core of the subunit, which is unaffected by hydration. A core type subunit model has already been suggested^{18,30}.

The DNA component of chromatin at the lower concentrations contributes scatter to the rings at 5.5 and 2.7 nm, each chromatin subunit possibly contains 1.5-2 turns of a coil of DNA (pitch 5.5 nm) wound on the outside of the histone core. Other DNA folds are however possible.

A schematic representation of our model is shown in Fig 6. A DNA subunit length of 205 base pairs could be accommodated in the model if the mean diameter of the DNA coil is about 10.6 nm. Since part of this DNA forms links between the subunit, the diameter of the DNA coil would be correspondingly reduced, depending on the length of these links. It is possible that the 170 base pair DNA unit observed¹⁸ forms part of the subunit, the additional 35 base pairs forming the link.

Very lysine rich histone H1, is not involved in the subunit structure giving the low angle rings⁷ and many studies have implicated H1 in a cross-linking role in chromatin. Further, the radius of gyration of chromatin is reduced after specific removal of H1 (ref 32), suggesting that this histone is on the outside of the chain of globular units. The constant value for the radius of gyration of chromatin in H₂O and D₂O proposed³², is not inconsistent with this model if both H1 and non-histone proteins were located on the outside of the DNA coil. It is now clear, however, that detailed neutron scatter studies are required at about 44% D₂O.

Chromatin is thought to consist of a string of such globular units with unknown orientations. The packing of such units in the chromosome is mediated through protein (probably H1) interaction, similar interactions probably take place between isolated chromatin subunits. In this case, therefore, the diffraction pattern of the isolated units would be similar to those of native chromatin since a diffraction peak corresponding to the lowest angle ring would arise from simple aggregation of the subunits³³.

An attractive general hypothesis concerning the control of chromosome structure through the cell cycle is that chemical modification of histones are involved. With only five major histones a chemical modification of any histone will modify the interaction of that histone with DNA throughout the genome. Further, these chemical modifications affect the state of charge of basic residues, or of serines and threonines, which are located in or close to the basic segments of the histone molecules. It may be significant that in the model proposed above the basic segments of the histones are complexed with DNA on the outside of the globular unit and are thus accessible to enzyme modification or enzyme attack. Phosphorylation of histone H1 has been implicated in the initial stages of the process of chromosome condensation and control of cell division^{31,34,35}.

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letters to nature

Nature of Her X-1

IYENGAR *et al*¹ have reported observations of hard X rays from Her X-1, which seem to set an upper limit of 10% on the pulsating component in the energy range 20 to 45 keV. I offer a simple explanation that does not require the additional source of X rays postulated by Iyengar *et al*.

In a strong magnetic field with $\omega \ll \omega_B$ (where ω is the photon frequency and $\omega_B (= eB/m_e c)$ is the electron gyro-frequency) the Thomson scattering cross section is reduced by $\sim (\omega/\omega_B)^2$ from its zero-field value for electromagnetic waves with E-vector pointing perpendicular to the field lines (because the electrons are constrained to oscillate along the field lines). In a magnetised plasma with $\omega \ll \omega_B$, the E-vector of the 'extraordinary' mode will essentially always be perpendicular to the magnetic field, whereas for the 'ordinary' mode this will be the case only for propagation along the field lines² thus the reduction in the Thomson scattering cross section occurs in general only for the extraordinary mode. I now refer to the model widely used to explain the X-ray pulsing from members of binary systems (specifically Her X-1 and Cen X-3), in which a rotating neutron star with a non-aligned magnetic field accretes material from its companion, the magnetic field channelling the infalling matter toward a 'hot spot' near the surface of the neutron star³⁻⁶. As I show elsewhere⁷, the gravitational energy carried by the infalling ions is transferred to the electrons in a stationary background plasma through Coulomb collisions, these electrons then produce the bulk of the observed X-ray emission, the mechanism depending on the strength of the magnetic field at the neutron star surface, B_0 . Should this field be so strong that $\hbar\omega_{B_0} \sim kT_e$, then the quantisation of the electron orbital energy must be taken into account. In particular, if $kT_e < \hbar\omega_{B_0}$ the only state energetically allowed to most electrons is the ground state, so that these electrons are constrained to move parallel or antiparallel to the field lines, and cannot emit cyclotron radiation.

It may now be argued that photons with frequencies $\omega \ll \omega_{B_0}$, produced at the base of the accretion funnel, will be markedly beamed in a direction perpendicular to the field lines. They are emitted by nonrelativistic electrons undergoing one-dimensional bremsstrahlung, so that the original radiation pattern is dipolar with maximum intensity perpendicular to the field lines (note that the photon frequencies far exceed the plasma frequency, and that the emitted photons are predominantly linearly polarised and in the ordinary mode), and the scattered radiation

pattern will be similar. Furthermore, even though the Thomson scattering cross section is reduced for both modes propagating along the field lines, a photon is not likely to escape upward through the accretion funnel, not only because it encounters far more material in that direction, but also the mean free path against Thomson scattering rapidly decreases in that direction because of the fall-off of the magnetic field, so that the photon tends to random walk downwards. Indeed, if one assumes that within the accretion funnel the plasma flows along the field lines at the free-fall velocity, then the steady state continuity equation yields

$$n(r) \sim n(R)(r/R)^{1/2} [B(r)/B_0] \quad (1)$$

where r denotes radial distance from the centre of the star and R the radius of the star, so that for reasonable magnetic fields $B(r)$ the fall-off of density in the accretion funnel, $n(r)$, is sluggish compared to that in, for example, the emitting region itself⁷. On the other hand, above the emitting region, the scattering cross section increases as $\sim (\omega/\omega_B)^2$, so that the photon mean free path decreases as

$$l_{\parallel}(r) \sim l_{\parallel}(R)(R/r)^{1/2} [B(r)/B_0] \quad (2)$$

until $B(r) \rightarrow \omega m_e c/e$ and the Thomson scattering cross section relaxes to its zero-field value, at which point the photon mean free path begins to increase inversely as $n(r)$. As an example, for a dipole field $B(r) \propto r^{-3}$, one has $n(r) \propto r^{-5/2}$ and $l_{\parallel}(r) \propto r^{-7/2}$. The beaming will be further enhanced through the conversion of ordinary into extraordinary mode photons in some scatterings, the latter then escaping easily if they are directed approximately perpendicular to the axis of the accretion funnel (A more detailed investigation of photon diffusion through plasma in a superstrong magnetic field is being carried out by M. Rosenberg and Y.-M. W.) It is then clear that the radiation must emerge near the base of the accretion funnel in a markedly fan-shaped beam (exhibiting, incidentally, substantial linear polarisation).

On the other hand, at higher frequencies, that is for $\omega \rightarrow \omega_{B_0}$, the electrons are no longer constrained to move one-dimensionally while the Thomson scattering cross section rises to its zero-field value for both modes of propagation, and the beaming should be at least partially smeared out (The magnitude of this effect will depend on, for example, the density gradient transverse as compared to that parallel to the axis of the accretion funnel). I assume that the pulsating component of the Her X-1 emission vanishes between 20

and 45 keV, and obtain a rough estimate of the magnetic field at the star surface by setting $20 \text{ keV} \lesssim \hbar\omega_{B_0} \lesssim 45 \text{ keV}$, from which $2 \times 10^{13} \text{ gauss} \lesssim B_0 \lesssim 4 \times 10^{13} \text{ gauss}$. Interestingly, Iyengar *et al* claim that the data for the entire energy range 2–80 keV can be fitted by a bremsstrahlung spectrum with a temperature of about 23 keV. On the basis of the above considerations, one would expect a bremsstrahlung spectrum below $\sim \hbar\omega_{B_0}$, although the shape of the spectrum above $\sim \hbar\omega_{B_0}$ is difficult to determine, one might expect a steep fall-off because of cyclotron emission.

I emphasise that the absence or near absence of hard X-ray pulses from Her X-1 claimed by Iyengar *et al* is as yet unconfirmed by any other group. Indeed, Holt *et al*⁸ find that the Her X-1 pulsed emission above $\sim 12 \text{ keV}$ is characterised by narrower peaks than that at lower energies (with no apparent difference in the ratio of pulsed to non-pulsed emission), in addition, they find a sharp cutoff in the (total) spectrum at $\sim 24 \text{ keV}$ which seems irreconcilable with the high energy data of Iyengar *et al*. If the surface field on Her X-1 is relatively weak, so that $\omega > \omega_{B_0}$ over the observed range of energies, then the dominant emission mechanism would be magneto-bremsstrahlung at a rate determined by the collision frequency (since the cyclotron decay time is far shorter than the Coulomb deflection time for an electron) giving rise to a soft energy spectrum⁷, and in this case the pulses at low energy might well be somewhat broader than those at higher energy because of the emission of soft X-rays above the star surface (for example, cyclotron emission by electrons spiralling down the accretion funnel).

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Model for 1.24 s X-ray pulses in Her X-1

THE 1.24 s X-ray pulses observed in the 2–20 keV region from Her X-1 are thought to arise from matter being accreted on to a rotating, magnetic neutron star. The pulse shape has been studied by several groups^{1–3}. More than 65% of the emission is concentrated in 0.4 of the duty cycle. The emission in this interval is usually double peaked, though it is sometimes single peaked¹ or asymmetrical^{2,3}. The pulse profile varies on time-scales of hours to days¹, and from pulse to pulse (my unpublished work with T. Chetani and R. Giacconi).

The pulsed emission is usually thought to be produced by a process analogous to that of radio pulsars. X-rays are emitted in a directed fan beam from the magnetic poles of the neutron star, which scans a circle in the sky as the star rotates. The Earth is in the line-of-sight of the beam only during a portion of each 1.24 s period. The double-peaked feature can be accounted for qualitatively by assigning particular values to the angle between the rotation axis and the magnetic axis (angle α), the angle between the rotation axis and the line-of-sight (β), and the width of the emitted fan beam². This model relies on two important assumptions: highly anisotropic X-ray emission from the base of the accretion column (beam width $\approx 25^\circ$), and constant luminosity at the source. A theory of highly beamed emission caused by synchrotron processes

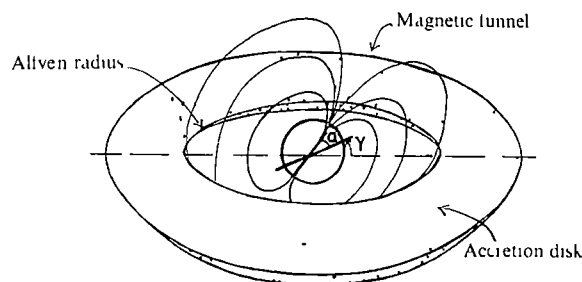


Fig 1 Diagram of Her X-1 with accretion disk. Magnetic funnel is shown here out of accretion plane. Neutron star is not to scale.

has been developed in support of this model⁴. But it is not clear that some proposed alternative models^{5–7} can account for the requisite narrow beam width. Beaming in these models is difficult if the magnetic moment of the neutron star is greater than $2 \times 10^{29} \text{ gauss cm}^3$ (ref. 8). The first assumption is thus open to question.

The second assumption is called into question by the possibility that the amount of matter falling into the accretion column will vary within each period. The accreting matter spirals into the neutron star in a thin plane until it is caught by the magnetic 'funnel' at the Alfvén radius⁸ ($R_{\text{Alf}} \approx 10^8 \text{ cm}$), from which it is accelerated directly on to the surface. For most choices of α and the angle between the rotation axis and the accretion plane (γ) the magnetic funnel will not lie continuously in the accretion plane but will sweep in and out of it during each rotation (Figs 1 and 2). Since the plasma density in the accretion plane is much greater than the density of matter distributed generally around the magnetosphere, one would expect well defined spurts of dense plasma to fall down the accretion column as the funnel passes through the plane. If these density variations arrive at the base of the accretion column intact, the X-ray emission will be pulsed. For many values of α and γ the funnel will pass through the accretion plane twice in each period, thereby providing for the double-peaked pulse structure (Fig. 2a).

So I propose that the 1.24 s pulses originate from luminosity variations at the source arising from time-varying accretion rather than from the passage of a beam of constant intensity past the Earth.

Unlike the conventional model, my model does not depend on any particular emission mechanism or beam width, puts no restriction on β , and takes cognisance of the anisotropic distribution of accreting matter around the magnetosphere.

This model is only feasible in certain conditions. First, as with the standard model, α cannot be 0° . This condition is met by most known neutron stars. Second, in order for the magnetic funnel to move in and out of the accretion plane, γ must not be 90° . Though it is frequently claimed that $\gamma = 90^\circ$, my condition will hold if the rotation axes of Her X-1 and HZ Herculis, the companion star, are not aligned. They might have been misaligned before the collapse of Her X-1 or γ might have been changed during the collapse. It has further been suggested that if HZ Her were once illuminated unevenly by X-rays from Her X-1, the accretion disk would permanently lie out of the orbital plane, resulting in $\gamma \neq 90^\circ$. Two forces might be thought to force the rotation axis perpendicular to the accretion disk: relativistic frame dragging and torque exerted by the accreting matter. The frame-dragging effect will significantly alter the orientation of the disk only if $R_{\text{Alf}} \lesssim 2 \times 10^7 \text{ cm}$ (ref. 10). The torque will be directed against the magnetic axis rather than the rotation axis. Thus, unless the magnetic and rotation axes align themselves or the neutron star wobbles, the magnetic funnel will still sweep in and out of the accretion plane during each rotation.

Next, it is assumed that the movement of the magnetic funnel through the accretion plane does not seriously distort

the structure of the accretion disk. This requirement can be verified only when more is known about the interactions at the Alfvén radius.

Finally, this model imposes some conditions on the properties of the plasma in the accretion column. Limits on turbulence and convection during the descent are required so that variations in the density of the infalling plasma are not smoothed out beyond the timescale of features observed in the Her X-1 pulse profile (rise time ≈ 50 ms). Since the total free-fall time from R_{Alf} to the surface of the neutron star is ≈ 100 ms, this condition would be violated only by very largescale effects in the column or by significant delays at shock fronts or deceleration zones. The model further demands that each spurt of plasma cool within ≈ 50 ms. This has been substantiated by preliminary calculations⁸.

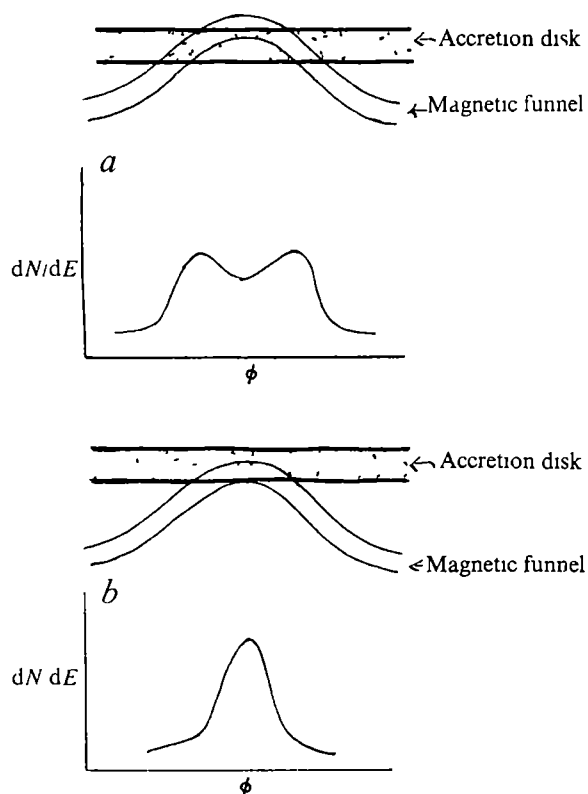


Fig. 2 Relative position of magnetic funnel and accretion disk as a function of phase, and the resulting pulse shape *a*, double-peaked pulse, *b*, single-peaked pulse after precession of the neutron star or accretion disk

None of the conditions listed here seem insurmountable, though several may warrant further investigation.

Since the rotation axis and the accretion plane are not perpendicular in this model, either the neutron star or the accretion disk will be precessing. Precession has been proposed by several authors as the source of the 35-d periodicity of Her X-1^{9,11}. Evidence for variation in the shape of the 1.24 s pulse across the 35-d cycle would provide strong evidence for the present model. Qualitatively, it predicts the secondary minimum to be more pronounced in the middle of the 9-d 'on' state, and more single-peaked pulses to be observed at the beginning and end of each 'on' state (Fig. 2*a* and *b*).

Short term variations in pulse shape and intensity ($\approx 1-10$ s) observed in Uhuru sightings (unpublished) of Her X-1 may be explained in this model in terms of fluctuations in accretion disk density. The fluctuation frequency is expected to be roughly equal to the orbital period at R_{Alf} , $t \approx 2\pi(GM/R_{\text{Alf}}^3)^{1/2} \approx 1$ s (ref. 10), as observed. This model, like the standard

model, cannot easily account for reported asymmetries in the pulse profile.

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Cosmological effects of primordial black holes

ALTHOUGH only black holes with masses $\gtrsim 1.5 M_{\odot}$ are expected to result from stellar evolution¹, black holes with much smaller masses may be present throughout the Universe². These small black holes are the result of density fluctuations in the very early Universe. Density fluctuations on very large mass scales were certainly present in the early universe as is evident from the irregular distribution of galaxies in the sky³. Evidence of density fluctuations on scales smaller than the size of galaxies is generally thought to have been destroyed during the era of radiation recombination⁴. But fluctuations in the metric of order unity may be fossilised in the form of black holes. Observation of black holes, particularly those with masses $M < M_{\odot}$, could thus provide information concerning conditions in the very early Universe.

One indication that many black holes exist at present in the Universe is the evidence that the average density of matter in the Universe greatly exceeds the observed density of matter. Application of the virial theorem to galactic clusters⁵ implies that the density of matter is at least five times the observed density. Measurements of the deceleration⁶ and positions³ of galaxies suggest that the density of matter may be larger than the observed density by a factor ~ 100 —perhaps enough to make the Universe closed, although these measurements are rather uncertain. On the other hand, there are reasons⁷ for believing that the observed deuterium in the Universe was formed in the early radiation era. This would place an upper limit on the free nucleon density during the first 15 min of the Universe. This upper limit on the nucleon density implies that the present-day matter density $\lesssim 6 \times 10^{-31} \text{ cm}^{-3}$, that is, ~ 10 times the observable matter density. This upper limit on the total matter density would be consistent with applications of the virial theorem to galactic clusters but would not be consistent with the above-mentioned measurements suggesting a higher density. If evidence for a cosmologically flat or closed Universe holds up then it follows that during the first 15 min most of the matter in the universe must have existed in some other form than free nucleons—in other words, black holes.

If many small black holes ($M < M_{\odot}$) exist at the present time then their presence may be revealed because they radiate electromagnetic radiation. Indeed, during collapse the metric will be changing rapidly on a time scale $\tau \approx 10^{-5} (M/M_{\odot})$ so that production of massless particles with energy of order h/τ is expected⁸. Thus masses smaller than about 10^{20} g will radiate X rays and gamma rays when they undergo gravitational collapse. Hawking⁹ has suggested that the emission of massless

particles can be interpreted by saying that black holes have a temperature $\sim 10^{-6} (M_{\odot}/M)$ K. This interpretation implies that black holes with masses as large as 10^{15} g would have radiated away all their mass by now. Davies and Taylor¹⁰ have, however, suggested that the emission of radiation from a black hole only takes place for a brief instant during the collapse and that only black holes with mass $\lesssim 10^{-4}$ g will radiate away a significant fraction of their mass. If they are correct then practically all radiation due to black holes would have been emitted in the very early Universe and at the present time would only show up as a possible contribution to the 3 K microwave background.

On the other hand, if Hawking's interpretation is correct then small black holes would have produced X- and gamma-radiation up to and including the present time. If the black hole mass spectrum is not varying too rapidly the result would be background radiation the spectrum of which started out as a continuation of the 3 K blackbody spectrum and rose slowly to a peak at an energy determined by the smallest black hole mass now existing. Observational evidence for Hawking's interpretation might be provided by a distortion in the 3 K blackbody spectrum for wavelengths < 1 cm or by a peak in the isotropic X-ray background above 10 MeV (corresponding to Hawking's estimate of 10^{15} g as the smallest black hole mass now existing). A flattening of the isotropic X-ray background spectrum at about 30 MeV has, in fact, been reported¹¹. If we identify the measured X-ray flux associated with this feature ($\sim 10^{-6}$ photons $\text{cm}^{-2} \text{s}^{-1} \text{keV}^{-1}$) with X-ray emission from black holes with masses in the neighbourhood of 10^{15} g then one arrives at a space density of these black holes $N_{\text{max}} \approx 10^{-52} \text{cm}^{-3}$. Because the present-day spatial density of galaxies is about 10^{-76}cm^{-3} we conclude that there may be as many as 10^{23} small black holes per galaxy. If we assume that the volume of our Galaxy is 10^{12}pc^3 then a region the size of our Solar System ($\sim 10^{44} \text{cm}^3$) might contain several small black holes. Of course, there are other possible interpretations^{12,13} of the 30 MeV feature in the X-ray background and therefore the actual spatial density of small black holes may be much less than N_{max} .

In order to calculate the contribution of small black holes to the total mass of the Universe one must make some assumption about the primordial black hole mass spectrum. The simplest assumption that can be made is that the number of black holes in each logarithmic interval of mass is the same

$$dN = N_0 dM/M \quad (1)$$

This assumption is consistent with the idea¹⁴ that perturbations of the metric in the early Universe should be independent of scale length.

With the mass spectrum (1) the total mass of primordial black holes is $N_0 M_{\text{max}}$ where M_{max} is the maximum mass of a primordial black hole. Taking $N_0 = N_{\text{max}}$ and a present-day mass density $\lesssim 10^{-30} \text{g cm}^{-3}$ gives $M_{\text{max}} \lesssim 10^{22} \text{g}$. This maximum mass for primordial black holes may be related to the Hubble mass at the time when black hole formation in the early Universe stopped. It is interesting that the Hubble mass was equal to 10^{22}g at some time during the hadron era ($t < 10^{-4} \text{s}$) when large density fluctuations are 'predicted' to occur in some theories of dense hadronic matter¹⁵. Conversely, identification of any of the cosmological effects we have discussed as being the result of small black holes should provide some information on the nature of dense hot hadronic matter. A search for evidence of small black holes in the Solar System might be very worthwhile. Indeed, the existence of small black holes in the Solar System might have considerable economic significance because small black holes would be very useful as power sources¹⁶.

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ATS-6 radio beacon experiment

SINCE ATS-6 was launched into a geostationary orbit at 94°W in late May 1974, measurements of the total electron content (TEC) using the Faraday polarisation-rotation¹ and group dispersive-delay techniques have been made at Fort Monmouth (40°18'N, 74°06'W). Comparison of TEC rate of change obtained by the two techniques yields the temporal variation of the integrated number of free electrons above the ionosphere. This variation indicates a flow of electrons from regions above the ionosphere into the ionosphere at night, while during the day the direction of flow is reversed. The rate of the electron flux is estimated using the continuity equation.

The total Faraday¹ rotation from the signal source to the observer is related to the total electron content by the expression

$$\begin{aligned} a &= (k/f^2) \int B \cos \theta N ds \\ &= (k/f^2) \int (B \cos \theta \sec \chi) N dh \\ &= (k/f^2) M N_1 \end{aligned} \quad (1)$$

where $k = 2.36 \times 10^{-5}$, M is the magnetic field factor (at 420 km), N_1 is the total ionospheric electron content, and $f = 140$ MHz. As B decreases inversely with the cube of the geocentric distance and the electron density decreases exponentially with altitude above F_2 (max) (~ 300 km), the rotation is heavily weighted near the Earth and is considered to provide electron content values below $\sim 1,200$ km.

Using the dispersive-group-delay technique², the phase of the modulation envelope between a carrier and its sideband is compared at two frequencies (nominally $f_{1,2} = 140, 360$ MHz with a sideband displacement of $\Delta f = 1$ MHz). Since the phase is insensitive to the Earth's magnetic field, this technique yields the number of electrons along the entire path from satellite to observer (N_T).

The differential modulation phase $\Delta\phi$, in degrees, is

$$\Delta\phi/360 = 40.3 \Delta f/c \sec \chi (f_1^{-2} - f_2^{-2}) N_T \quad (2)$$

where c is the speed of light *in vacuo*, and χ is the zenith angle.

The relative variation of the total electron content measured by the Faraday and group-delay techniques is shown in Fig. 1a and b at 15-min intervals for the time period 1600 EDT on July 3 to 0800 EDT on July 8. The temporal variations of N_1 and N_T were nearly parallel with most density variations observed on both curves. In general, $\Delta N_1/\Delta t$ and $\Delta N_T/\Delta t$ varied between $\pm 1 \times 10^{16}$ electrons m^{-2} per 15-min interval.

Large increases of total electron content, in response to two large solar flares, are prominent during the time period covered by Fig. 1. Between 0945 EDT and 1000 EDT on July 4, N_1 increased by $\sim 1.5 \times 10^{16}$ electrons m^{-2} , while N_T increased by $\sim 2 \times 10^{16}$ electrons m^{-2} . Since the content values in Fig. 1a and b are given every 15 min, the full increase of N_1 and N_T is not indicated there. Starting at ~ 0953 EDT, N_1 increased by $\sim 3.3 \times 10^{16}$ electrons m^{-2} in 3 min and then decayed to its figure value at 1000 EDT. At the same time, N_T increased by approximately

the same amount. On July 5 between 1730 and 1745 EDT, N_I increased by $\sim 1.9 \times 10^{18}$, while N_T increased by $\sim 2.3 \times 10^{18}$. The rapid increases started at ~ 1740 EDT with N_I increasing by $\sim 2.1 \times 10^{18}$ in ~ 6 min, N_T increased similarly.

The dispersive-group-delay technique measures the total electron content from observer to the geostationary satellite, whereas the Faraday technique yields the content only in the vicinity of the Earth (up to $\sim 1,200$ km). The difference between the two yields is the content above $\sim 1,200$ km, which is referred to as the plasmaspheric content, N_P .

It follows that the rate of change of the plasmaspheric content is

$$\Delta N_P / \Delta t = \Delta N_T / \Delta t - \Delta N_I / \Delta t \quad (3)$$

The rate of change $\Delta N_P / \Delta t$ at 15-min intervals is plotted in Fig 2a and b for the same time period as Fig 1. The absolute values of $|\Delta N_T / \Delta t| - |\Delta N_I / \Delta t|$ for either positive (N_I and N_T both increase) or negative (N_I and N_T both decrease) are indicated. A negative value for either of the two cases indicates that the N_I variations was larger than the N_T variation. This must indicate a flow of electrons between the plasmasphere and the ionosphere, which would change N_T by a lesser amount than the corresponding change in N_I .

Figure 2 shows that, for the most part, the changes of the plasmaspheric content at 15-min intervals were generally restricted to $\pm 0.5 \times 10^{18}$ electrons m^{-2} .

As expected the rate of change of N_T was more often than not larger than the rate of change of N_I (all positive values in Fig 2). We expect this because N_I is part of N_T , and any changes in N_I will be reflected in N_T . An interesting behaviour of $\Delta N_P / \Delta t$ occurred during the decay phase of the content on July 5-6, July 6-7 between ~ 2000 and 0430 EDT, when it was generally positive indicating that the total content was decreasing faster than the ionospheric content. Since loss of plasmaspheric content through recombination cannot account for the difference between $\Delta N_T / \Delta t$ and $\Delta N_I / \Delta t$, the only plausible

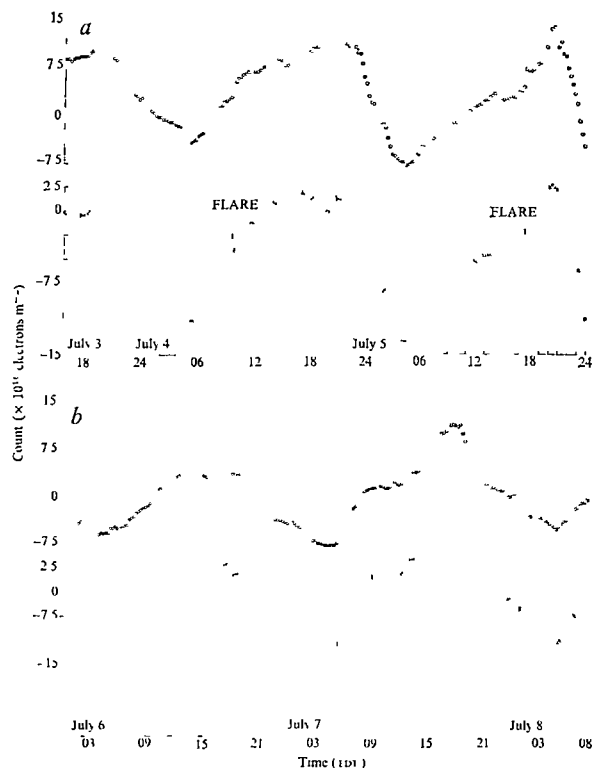


Fig 1 Relative variation of total electron content, N_T (○), and ionospheric electron content, N_I (×) at 15-min intervals 1600 EDT, July 3, 1974, to 0800 EDT, July 8, 1974, at Fort Monmouth, New Jersey

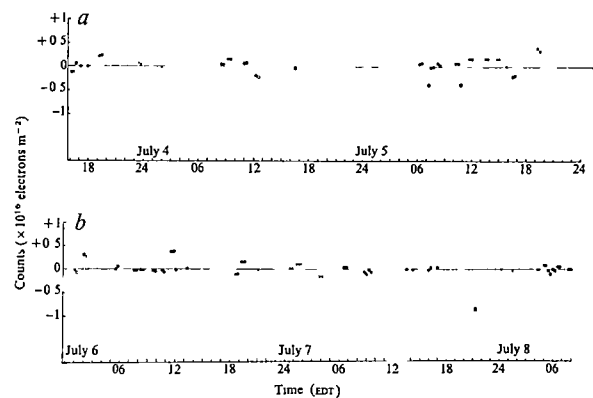


Fig 2 Variation of plasmaspheric electron content ($N_T - N_I$) ○, variation for $N_T, N_I > 0$, × variation for $N_T, N_I < 0$ A negative value indicates $N_I > N_T$

cause for the difference is electron flux from the plasmasphere to the ionosphere.

I suggest the following mechanism. The ionospheric electron content decreased sharply which resulted in equal decreases of N_T and N_I . With the corresponding fall in electron temperature, ionospheric layer collapse induced electron fluxes from the plasmasphere to the ionosphere. Although these fluxes did not alter the value of N_T , since they were still measured by the group delay technique, they did slow the rate of decrease of N_I because they reached lower altitudes where they were then measured by the Faraday rotation technique. Increases in total ionospheric electrons³⁻⁵ can be seen with the Faraday technique only when the flux rate exceeds the recombination rate, indicating an actual increase in ionospheric total density. But with the combination of Faraday and group-delay techniques, made possible by the radio beacon experiments of ATS-6, such fluxes may be determined even when their rate is smaller than the decay rate due to recombinations.

Assuming electron transport in the vertical direction only, the magnitude of the fluxes can be estimated.

The rate of change of N_I can be expressed by the continuity equation

$$\Delta N_I / \Delta t = q_I - L_I - F \quad (4)$$

where q is the production rate, L is the loss rate ($L = \int_{\text{Ionos}} \beta(h) N(h) dh$ where $\beta(h)$ is the loss rate coefficient) and F is the flux term. Similarly for N_T

$$\Delta N_T / \Delta t = q_T - L_T \quad (5)$$

The flux term has been omitted here since any fluxes between ionosphere and plasmasphere will still be measured by the group-delay method. Since production and loss are effective only at ionospheric heights, it follows that $q_T \approx q_I$, $L_T \approx L_I$, and therefore

$$\Delta N_T / \Delta t - \Delta N_I / \Delta t \approx F \quad (6)$$

using the results of Fig 2, the estimated fluxes are $\lesssim \pm 10.5 \times 10^{13}$ electrons $m^{-2} s^{-1}$.

Particle fluxes also play a role during the build-up phase. For example, between 0400 and 1400 EDT on July 6, $\Delta N_P / \Delta t$ was generally positive indicating that N_T increased faster than N_I . Electron production cannot contribute to an increase in N_P , but an increase in the particle temperature expands the ionospheric layers and thus causes electron fluxes from the ionosphere to the plasmasphere, increasing the content of the latter N_T .

reflects the increase in production in the ionosphere and electrons in the plasmasphere due to these fluxes. The rate of increase of N_1 is slower since the fluxes slow down the rate of increase by electron production.

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Effect of vorticity pollution by motor vehicles on tornadoes

THE recorded annual incidence of tornadoes in the United States has increased steadily and dramatically in the past four decades, by at least a factor of six¹. We have examined the thesis that the development of widespread motor vehicle traffic in the United States over the last 40 yr has perturbed atmospheric vorticity in such a way as to exacerbate the tornado incidence, principally by the introduction of cyclonic vorticity. Surprisingly, both the analysis and the evidence support the thesis.

Tornadoes depend on non-random vorticity in strong convection and motor vehicle traffic produces a unique anthropogenic input of non-random vorticity into the troposphere.

Motor traffic on the North American continent passes opposing traffic on the right. The thrust of each motor vehicle against the solid Earth is thus ordinarily to the right of that of the opposing traffic. To the extent that these thrusts result from momentum transfer to the atmosphere, a plethora of additive anticyclonic force couples act on the Earth's crust, and cyclonic torque is applied to the atmosphere. These effects persist and accumulate, limited only by the exchange of the atmospheric vorticity with the solid Earth, which according to our calculations occurs slowly. It is this anthropogenic addition to atmospheric cyclonic vorticity that might constitute a link between motor vehicle traffic and tornadoes. We are here neither contemplating the temporary turbulence in the wake of passing vehicles, nor the angular momentum stored in the vehicles underway, but rather the interaction of the opposed atmospheric momentum streams engendered by two opposed streams of traffic (Fig 1).

In the USA there are $\sim 2 \times 10^6$ automobiles and 6×10^5 trucks underway at any average moment². Cyclonic vorticity introduced into the troposphere by US traffic in one week is much greater than the Earth's vorticity, and annually is of the order of 10^5 – 10^6 times the vorticity involved instantaneously in a single tornado. Trucks and automobiles contribute equally. In our calculation we used an average spacing of 20 m between opposed traffic streams. The magnitude of the torque is of course dependent on this number, and recent increased spacing between lanes has increased the total torque, but a reasonable range of spacings would affect the torque by less than one order of magnitude.

In our model, the continent was initially enveloped in a tropospheric air mass, drifting west to east, that contained an overall cyclonic vorticity with substantial regions of no or anticyclonic vorticity. With the increase of motor vehicle traffic, the air masses were treated with increasing cyclonic vorticity, which cancelled much of the anticyclonic vorticity and produced (a), a more homogeneously cyclonic troposphere, (b), more numerous (and more dominantly

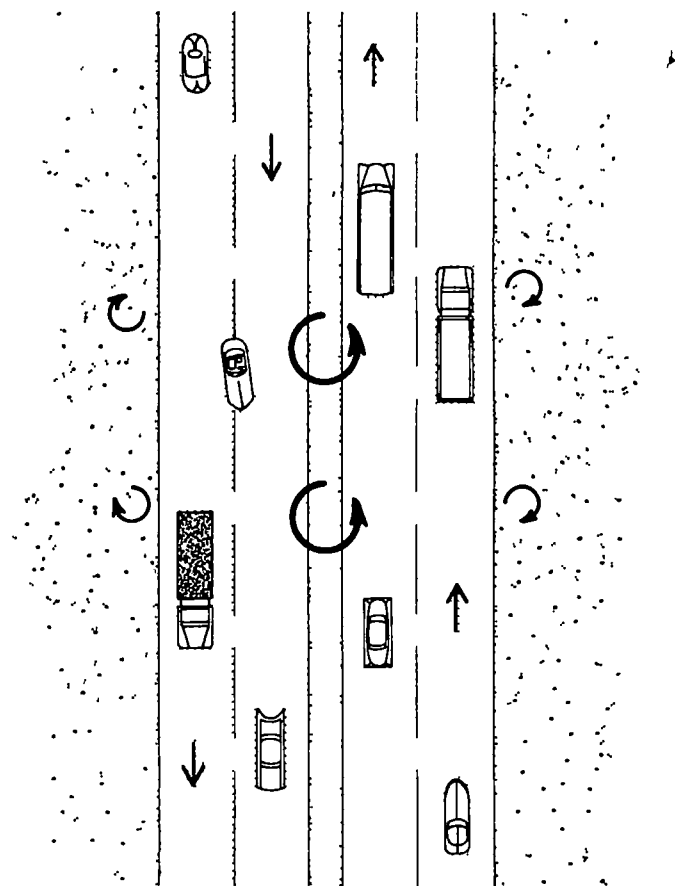


Fig. 1 "For they have sown the wind, and they shall reap the whirlwind" Hosea 8:7. The cyclonic vorticity introduced into the atmosphere is generated by the torque between the two opposing streams of traffic. The smaller side vorticities created at the periphery of the highway are boundary effects eventually acting at large distances (perhaps finally at the antipodes, where they are also cyclonic).

cyclonic) tornadoes, (c), a migration of the "centre" of tornado intensity to the east, and (d) secular and periodic variations of tornado incidence reflecting major changes in motor vehicle traffic (for example, weekly). The records indicate that these phenomena have occurred.

We have obtained the detailed record of all US tornadoes (15,234) reported between 1950 and 1873 in the 48 contiguous states³. The annual incidence shows an increase over the period of a factor of four. Changes in observation and reporting are undoubtedly serious and we have applied tests that are relatively independent of secular changes in reporting.

The annual centre of tornado intensity weighted by the area of each tornado path has varied considerably and has increasingly occupied the eastern portion of the continent in the second half of the record, as our thesis predicts.

Pollution by cyclonic vorticity should have lowered the relative incidence of anticyclonic tornadoes, and this has occurred. In the first half of the record (1950–61) 4 out of 11 events for which sense was recorded were anticyclonic, whereas in the second half, this was true for only 10 out of 123. A random selection of 11 events from a distribution similar to that of the second time period would have approximately a 7.5×10^{-3} chance of recording four or more anticyclonic tornadoes.

The data display a strong weekly periodicity.

Over the entire record the reported number of tornadoes on Saturdays is less than the daily average by more than 7.1 standard deviations (1,868 on Saturdays as against a daily average of 2,176). The statistical probability of this record occurring by chance from a random sample is $P < 10^{-8}$. This hebdomadal event dominates in 18 of the 24 yr and in most

states (Thirty-seven of the forty-eight states show a Saturday incidence below the average, $P \sim 10^{-4}$). Nineteen states show Saturdays incidence to be a minimum ($P = 1.5 \times 10^{-3}$).

We attribute this weekly periodicity to the periodicity of vorticity introduction by motor vehicle traffic. We attribute the general Saturday minimum to the fact that most truck and commuter traffic ceases on a Friday, and to the unidirectional character of the weekend flow, both in and out of urban areas, which probably introduces mainly a momentum stream into the atmosphere on Saturdays, generating net cyclonic vorticity later and downwind by interaction with the momentum stream of the traffic returning on Sunday. Other effects such as a weekend diminution of heat or particle production by industry and motor vehicles or of the efficiency of reporting are difficult to reconcile with average tornado incidence on Sundays in most states.

We would expect the strongest hebdomadal fluctuations to occur in states dominated by fresh, relatively unsullied oceanic air masses and thus influenced more sharply by the immediate input of vorticity. Indeed, coastal states display an enhanced weekly cycle with 12 of the 21 coastal states showing the Saturday incidence of tornadoes to be a minimum, and 19 of the 21 showing Saturdays to be below average in tornado occurrence (as opposed to seven showing Saturday minima and 18 showing Saturdays below average in the 27 interior states). These statistics for the special class of coastal states have 0.04 probability of being a chance selection from the statistics of all states. This test of our thesis should be independent of general reporting peculiarities, and so is crucial evidence in its support.

Additionally, as the thesis predicts, far western coastal states display a special selection of the statistics of all coastal states with minima for both Saturdays and Sundays. No far western state is subject to many tornadoes (also perhaps in support of our thesis) and, in the extreme case of the state of Washington, of the 25 tornadoes reported in the past 24 yr, none occurred on Saturday or Sunday ($P \sim 2.2 \times 10^{-4}$).

Vorticity is a unique requirement for the formation of tornadoes and hurricanes, and motor vehicles are the only man-made source of non-random vorticity we know. From our initial findings we provisionally assign an increase in reported US tornadoes to vorticity pollution by motor vehicles. If this is the case, mankind, excluding mainly the British, may need to take responsibility for yet another deleterious environmental effect. But vorticity pollution could be turned to benefit. If the obvious turnabout were made, there might well be an immediate and secular decrease in tornadoes, perhaps to levels below the natural intensity, duration and frequency.

Regardless of the veracity of our thesis, some anthropogenic effect clearly has resulted in about 300 fewer tornadoes reported in the US on Saturdays than on other days of the week, in the past 24 yr. If this results from the reduction of some human activity on Saturday (other than tornado reporting), then at least 14% of US tornadoes are under man's control. The total of man's effects could, of course, be much larger since an accumulating diffuse reservoir of vorticity might account for more of the long term (600%) increase in tornado incidence (or even changes in hurricane incidence, path and intensity) in the Northern Hemisphere.

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Diachronism of depositional and diastrophic events

ONE of the fundamental principles of geology—'Stille's legacy'¹—is the concept that there is a wide contemporaneity of geological events, both tectonic and sedimentary. The concept received notable attention 25 yr ago and raised a scientific controversy². It is now of interest again because of the present tendency to assume as a causal mechanism of mountain building the relative motions between plates. On the basis of geological evidence it has been shown³ that the diastrophic events of the western Mediterranean do not occur at the same time in the different mountain chains, because collisions between plates are diachronous. Detailed stratigraphical analysis of the units within the Maghrebian Chain⁴ (from Gibraltar to Calabrian Arc) indicates that, in addition, the initiation of geological events varies from place to place in the same orogen⁵.

The Maghrebian Fold Belt was produced largely by the tectonic deformation of three groups of Oligo-Miocene flysch (the 'tectonic triad of flysch types'⁵) (see Fig. 1).

One group is represented by a Nile Cone-type^{5,6} 'passive margin' sequence which terminates in the upper part with an alternation of quartz-rich sandstones and iron-rich silty clays. In Sicily these African-derived⁷ miogeoclinal quartzose deposits derived from Africa, comprise typical submarine fan facies (the 'external' Nubian Flysch and the 'internal' Numidian Flysch) and abyssal plain facies (the Malia Flysch). The basal strata of these units are transgressive northwards over multicoloured marls (scaglia-type formation⁸) and clays (*Argille varicolori*⁵) interpreted as deep-water basinal facies. The northward fan progradation occurred with a biostratigraphically estimated rate of about 10–20 km Myr⁻¹ (Fig. 2).

The second contemporaneous flysch group (Hellenic Trench-type suite^{5,6}) comprises marly-arenaceous, compositionally-immature turbidites (Tusa Flysch). The marls are homogeneous ('homogenites') and probably originate from ponding by turbidity currents^{5,6}. The sandstones are lithic (clastics from granitic-metamorphic and sedimentary sources) with sometimes significant andesitic components⁸. The Tusa Flysch sequences constitute successive thrust sheets, each sheet less than 800 m thick and progressively younger southwards. This tectonic juxtaposition is inferred to be the result of subduction under the Calabrian Massif of original trench-filling sediments^{3,5}. Migration of the trench axis occurred at only about a fifth of the rate of progradation of the Nile Cone-type flysch (Fig. 2).

The third member of the 'triad' (the Crete Basin-type flysch⁵) is a thick, tectonically undeformed, arenaceous sequence which is transgressive on the Calabrian Massif in the internal (northern) zones and overlies the tectonically deformed Tusa trench-fill deposits in the external (southern) zones. This neo-autochthonous sedimentation (Reitano-Capo d'Orlando Flysch) is believed to be an arc-trench gap accumulation⁸ that advanced progressively southwards over the deformed trench deposits accreted to the front of the Calabria microplate^{3,5}. Sicilian

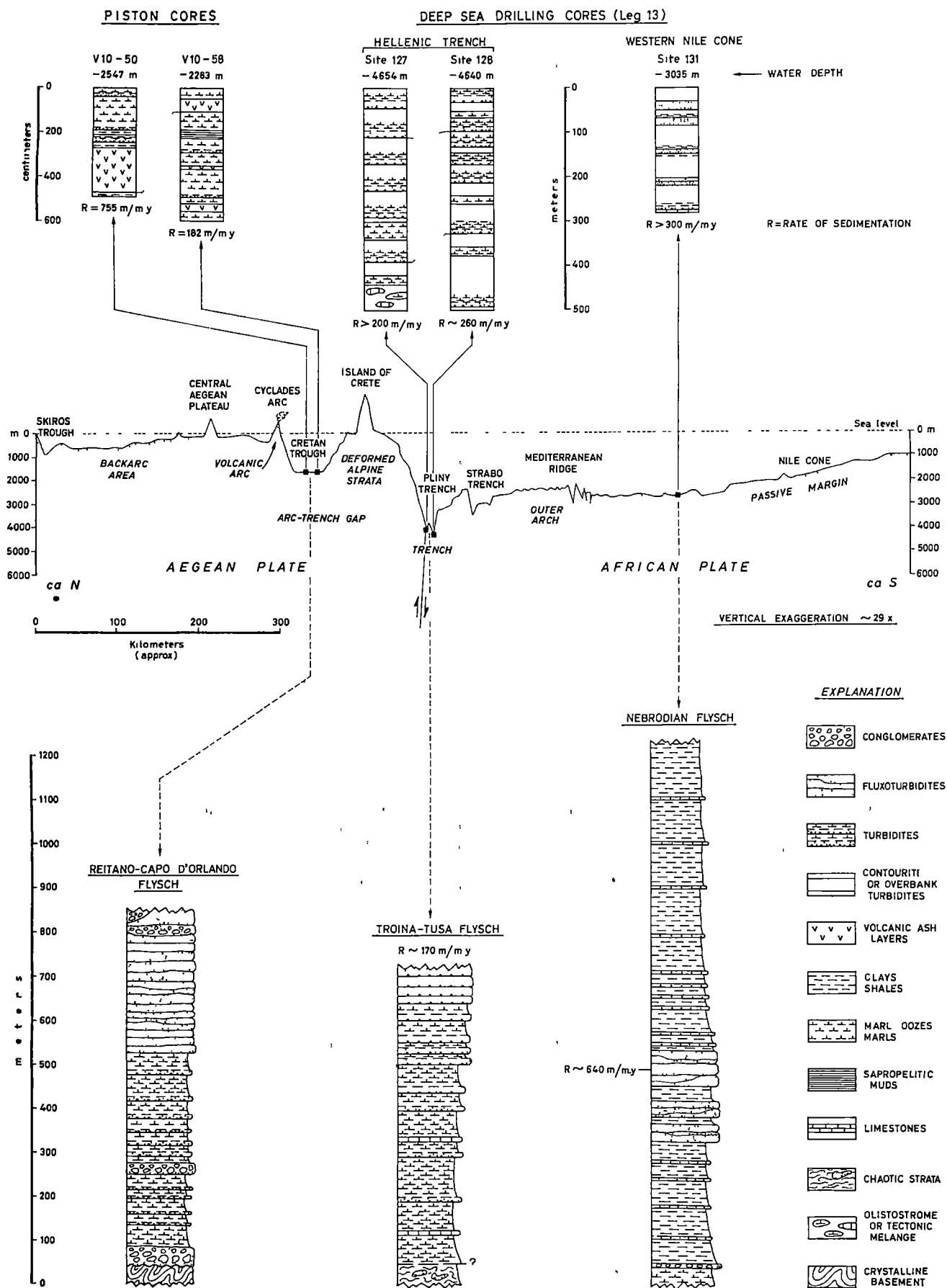


Fig. 1 The 'tectonic triad of flysch types' characteristic of a contracting basin, showing the present day (factual) and the ancient (inferred) tectonic settings for the three litho tectonic assemblages. In the upper part, the Quaternary sedimentary columns of the JOIDES cores⁶ and two Vema piston cores¹² were projected on to a bathymetric profile crossing the eastern Mediterranean and the southern Aegean at approximately 25°E¹³. The lower part shows generalised columnar sections of the three equivalent Oligo-Miocene flysch groups which crop out in the Sicilian part of the Maghrebain Chain.

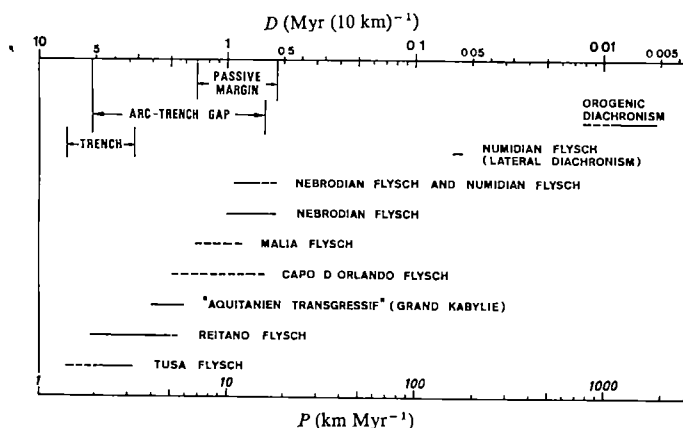


Fig 2 Biostratigraphic estimates of the transgression of basal strata of the Oligo-Miocene flysch and of orogenesis in Sicily. Depositional events—trench (Tusa Flysch) and arc-trench gap sedimentation (Reitano-Capo d'Orlando Flysch)—are characterised by a greater diachronism than passive margin deposition (Nebrobian, Numidian and Malia Flysch). The migration of the crustal suturing along the Maghrebain Chain is near the boundary of the time resolution at present achieved with planktonic foraminiferal zonation P , the rate of progression in km Myr^{-1} , and D , the diachronism coefficient in Myr (10 km)^{-1} , are new parameters proposed⁶ for a better quantitative evaluation of the magnitude of diachronism. Using these parameters 'bio-isochron lines' can be traced on geological maps, allowing estimations of relative past positions of passive and active continental margins.

data indicate a time span of only 3–4 Myr for a single 'tectogenetic cycle' which includes syntectogenetic trench sedimentation, tectonic deformation and the beginning of deposition of the arc-trench gap cover. In northern Morocco, between Tangiers and Ceuta, there is a spectacular juxtaposition of successive elongate thrust belts comprising Hellenic Trench-type flysch (Beni-Ider) and Numidian Flysch, and probably caused by the progressive accretion of deposits deformed in eastward facing subduction zones (F. Guerrero and W. B. F. Ryan, personal communication).

As in the case of the depositional events, the timing of orogeny is not synchronous everywhere in the Maghrebain Chain. Collision and suturing of the northern microplates and the North African continental margin occurred about 1 Myr earlier in Algeria¹⁰ (late Burdigalian) than in Sicily⁵ (early Langhian). The eastward migration of the locus of collision was accompanied by the termination of Numidian deposition. The most likely reason for this diachronous orogenesis seems to be either a non-parallelism between the approaching continental margins or an oblique closing of the flysch basin with some component of transcurrent motion.

In view of these data it seems difficult to maintain the classical concept that the Maghrebain nappes originated primarily as a result of centrifugal gravitational sliding away from an uplifted Tyrrhenian zone¹¹. The noncontemporaneity of sedimentary and diastrophic events in different parts of the same orogenic belt also brings into question the validity of the rigid formalism of traditional (proto) stratigraphical philosophy (that is, the platonic idea of stratotypes). It seems that a dynamic (sedimentological-ecological) approach would be more appropriate.

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Efficiencies of electrolytic and thermochemical hydrogen production

Two methods not involving fossil fuels for manufacturing hydrogen have been suggested as a basis for an eventual hydrogen economy: first, electrolysis of water using nuclear (or ultimately solar, geothermal or thermonuclear) electricity and, second, thermochemical cycles using nuclear heat, in general from a high temperature reactor^{2,3}. In the thermochemical processes, a series of chemical steps is envisaged that will allow the differential splitting of the H_2O molecule by means of reactions at different temperatures and with appropriate thermodynamic requirements to give high efficiency. The sequence of reactions, ideally of gas-solid type, acts as an adsorption-desorption-cycle heat engine. For economical hydrogen production, high thermal efficiency combined with low capital investment cost is necessary. The question is whether a system constructed on the basis of a sequence of chemical reactions will be more efficient (and ultimately less costly) than one based on a heat engine producing electricity, followed by electrolysis. Here I shall examine briefly some aspects of the efficiency of both approaches.

In the electrochemical case, the overall thermal efficiency is relatively simply defined as

$$e_t = \Delta H^0 / Q \quad (1)$$

where ΔH^0 is the standard heat of reaction for the oxidation of hydrogen to water under the conditions of use and Q is the total quantity of heat needed to effect the breakdown of 1 mol of water. If the electrolysis is reversible, that is, if the electrode processes (under conditions of no net reaction) with the reactants and products in their standard states have an exchange rate that is much higher than the desired net rate of breakdown, then, Q will be given by

$$Q = x\Delta G_t^0 + T\Delta S_t^0 \\ = x\Delta G_t^0 + (\Delta H_t^0 - \Delta G_t^0) \quad (2)$$

where ΔG_t^0 , ΔH_t^0 and ΔS_t^0 are the standard free energy, heat of formation and entropy of water at the temperature of electrolysis and x is the factor for the conversion of heat into (electrical) work. In general, liquid water will be used as feed-stock, therefore the ΔG_t^0 and ΔH_t^0 terms in Q will be those for liquid water. By contrast, the ΔH^0 term may be either for gaseous or liquid water, depending on whether or not condensation takes place during use of the heat.

In a real electrolyser, three types of loss occur, all of which add ΔG terms. The first results from the fact that the reactions at the electrodes have finite rates (overpotential). This is particularly so for the case of the oxygen electrode at normal temperatures. This overpotential is given by the Butler-Volmer equation in the most general case. The equation must, however,

be modified to take account of the effects of changing effective electrode area and of changing reactant concentration, which result from the remaining two losses—current distribution stemming from local resistance, and reactant depletion because of mass-transport effects (for example, the formation of product gas bubbles). In addition, irreversible energy loss occurs because of resistive (IR) drop throughout the system. Altogether, the additional free energy that must be provided to decompose water is conventionally represented by

$$\Delta(\Delta G_t) = \Delta G_\eta + \Delta G_{diff} + \Delta G_{IR} \quad (3)$$

where ΔG_η is the overpotential, calculated from the ratio of the net and exchange (equilibrium) rates of reaction using the Butler–Volmer equation, assuming the whole surface of the electrode to be active, ΔG_{diff} is the effective diffusion loss, and ΔG_{IR} is the loss attributable to the IR drop. For relatively small displacements from equilibrium, the ΔG_η and ΔG_{diff} terms linearise, so that the combined $\Delta(\Delta G)$ term is of pseudo-ohmic type. An expression for an uncomplicated single-electrode process under these conditions is given by

$$\Delta(\Delta G_t) = \iota \left[RT \left(\sum_n \frac{1}{\iota_{0j}} + \sum_m \left| \frac{1}{\iota_{Dk}} \right| \right) + nFR' \right] \quad (4)$$

where ι is the rate of decomposition (current per unit area), R is the gas constant, ι_{0j} is the equilibrium reaction rate of the j th reaction step, ι_{Dk} is the diffusion-limited rate of the k th step, and R' is the internal electrical resistance per unit area. In general only one step in each case will predominate with regard to diffusional and kinetic limitations, however, if the limiting steps occur more than once in the overall process, the $1/\iota$ terms must be correspondingly summed over the number of times they occur.

In general, for electrolysis at low temperatures $\Delta G_t^0 + \Delta(\Delta G_t)$ will exceed ΔH_t^0 , since under these conditions kinetic and diffusional limitations are considerable and, in any case, the value of $\Delta H_t^0 - \Delta G_t^0$ is small (~ 12 kcalorie for liquid water at ambient temperature). All the energy required for water decomposition will thus be free energy in the form of electricity. At higher temperatures, ΔG_t^0 falls rapidly (ΔG_t^0 for water vapour ~ 44 kcalorie mol^{-1} at 700°C) and the kinetic and diffusional terms become less important, so that in principle only a part of the energy used need be electrical work, the rest being provided by heat.

For the thermal method of splitting water, which involves the use of a series of chemical cycles devised in such a way that the ΔG^0 in each reaction is close to zero, thermal efficiencies are much less easy to define in detail. In general, if one assumes a perfect system with infinitely rapid reactions (complete reversibility) with no heat losses, then the efficiency is given by an expression similar to equation (1), summed over all the n reaction steps to be used in the cycle, that is

$$e = \Delta H^0 / Q_{rev} = \Delta H^0 / [x \sum_n \Delta G_{t,j}^0 + \sum_n (\Delta H_{t,j}^0 - \Delta G_{t,j}^0)] \quad (5)$$

where the j e terms refer to the j th step⁴. If the reactions involve separations, particularly of gaseous components, an irreversible work of separation corresponding to $T\Delta S_m$, where ΔS_m is the entropy of mixing of the components, must be added to Q_{rev} (ref. 4). This is, of course, directly avoided in electrolyte procedures. For this reason, cycles with gas–solid reactions involving only one gas are to be preferred where possible. In addition, inevitable heat losses that cannot be partially used as work will occur in the processes. Hence Q is given by

$$Q_{rev} + x \sum T \Delta S_m + \text{heat losses}$$

So far in the literature, efficiencies for the thermal cycle

method of generating hydrogen have been calculated on the above basis. In several cases (see ref. 5) x , the factor for the conversion of heat into work, has even been ignored (see, however, refs 4 and 6).

All such calculations regard the processes as ideal—each step is not only at equilibrium but is infinitely rapid compared with the net rate of the reaction. This situation is obviously idealised and corresponds precisely to ignoring the overpotential factors in electrolysis. As in the latter, free energy losses occur because of the net rate of the processes resulting from three similar causes: kinetic, diffusional and frictional. The kinetic limitations of each step are given by the chemical equivalent of the Butler–Volmer equation, namely the Marcelin–De Donder equation⁷. This may be expressed in the form

$$k = k_0 [\exp(\Delta A / RT) - (\Delta A' / RT)] \quad (6)$$

where k is the net rate of the process, k_0 is the equilibrium rate and ΔA , $\Delta A'$ are the displacements of chemical potential of the reactants and products from equilibrium (affinities) corresponding to overpotential in the electrochemical case.

In general, the reactions chosen for thermochemical cycles will be, in the optimum case, solid–gas reactions, in which the chemical potential of the reactants may be taken to be independent of the amount of material reacted. In these conditions, the measured (out of equilibrium) rate of reaction, on which process scaling is based, will be of the same order as that for the equilibrium rate. For a net rate close to this value, the minimum value of ΔA will be of the order of $1.3RT$ for each separate reaction step. Summed over all steps for a four-step process at an average temperature of 800 K , this represents 8.3 kcalorie of pumping work. Taking into account the x factor and system inefficiencies (viscous losses, concentration gradients), the total extra energy input may amount to 30 kcalorie per mol hydrogen as a reasonable minimum. Similar considerations apply to diffusion-limited steps, whether they occur in the chemical steps or in component separation. The latter will introduce additional work terms that must be added to the separation work. This extra energy can present a serious additional fall in overall efficiency. For example, a process with a reported estimated efficiency (hydrogen low heating value) of 45% (ref. 6), after all reversible work and heat losses are accounted for, will have a real maximum efficiency of only about 36% if one considers only the irreversible losses in the chemical steps. If the irreversible losses in separation steps are considered, the estimated maximum efficiency may approach 30% . This point illustrates the fact that irreversible thermodynamic considerations cannot be ignored in calculating the efficiencies of thermochemical cycles in which affinities must be summed over several steps, giving typical requirements for total extra work which may be greater than those for separation work. It seems, in fact, that, when all factors are taken into account, processes based on thermochemical conversion may prove to be less efficient than projected electrolyzers, both processes being supplied from a heat source at the same temperature.

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Enhancement in thermonuclear reaction rates in a dense plasma

For laser-driven fusion, a number density of the order 10^{26} cm^{-3} is usually envisaged^{1,2}, here I consider the effects of the presence of a medium on the nuclear reaction rates³ and show the feasibility of enhanced fusion rates when a certain condition is met. The results are applicable both to controlled thermonuclear reactions and to astrophysical conditions.

For a head-on collision of two particles carrying charges $Z_1 e$ and $Z_2 e$ in an electron-ion plasma with dielectric constant $\epsilon(\mathbf{k}, \omega)$, the Coulomb interaction is modified to⁴

$$V(\rho) = (4\pi Z_1 Z_2 e^2 / \nu) (2\pi)^{-3} \int d^3 \mathbf{k}_\perp \int_{-\infty}^{\infty} d\omega \times \\ \{ [k^2 \epsilon^2(\mathbf{k}, \omega)]_{k_z = \omega/\nu} \}^{-1} \exp \{ i(\omega \rho / \nu) \} \quad (1)$$

($Z_1 = Z_2 = 1$ for a deuterium-tritium (DT) mixture) Here, ν is the velocity of the incident particle which is taken to be along the z direction $\mathbf{k} = (k_\perp, k_z)$, and $\rho = z - \nu t$ is the distance between two colliding particles.

The ion velocity ν of interest usually far exceeds the ion thermal velocity $\nu_T = (3k_B T / M)^{1/2}$, where T is the ion temperature and M is the ion mass (For a DT mixture, M is taken to be a suitably averaged ion mass) Furthermore, when the electron temperature T_e exceeds T so that $\nu \ll u_T$ (where $u_T = (3k_B T_e / m)^{1/2}$ is the electron thermal velocity), we have

$$\epsilon(\mathbf{k}, \omega) \simeq (k_D / k)^2 + \{ 1 - [\Omega_p / (\omega + i\delta)]^2 \} \quad (2)$$

where $\delta \rightarrow 0+$, $k_D = (4\pi n_e e^2 / k_B T_e)^{1/2}$, and $\Omega_p = (4\pi n_e e^2 / M)^{1/2}$. (n_e is the number density of the electrons) For $(k_D \rho)^2 \ll 1$, we have

$$V(\rho) \simeq (Z_1 Z_2 e^2 / \rho) \{ 1 - (k_D \rho) [\alpha \lambda(\alpha) + \theta(1 - \alpha^2)(1 - \alpha^2)^{1/2}] \} \quad (3)$$

Here

$$\lambda(\alpha) = (\pi/2) - \theta(1 - \alpha^2) \arctan[(1 - \alpha^2)^{1/2} / \alpha]$$

with $\theta(x)$ being the unit step function [$\theta(x) = 1$ for $x > 0$ and zero otherwise] and $\alpha = 3^{1/2}(\nu_s / \nu)$ where $\nu_s = (k_B T_e / M)^{1/2}$ is the ion sound velocity.

Since the factor $[\alpha \lambda(\alpha) + \theta(1 - \alpha^2)(1 - \alpha^2)^{1/2}]$ does not exceed $\pi/2$, the dynamic screening at a small distance may be ignored. But the second derivative of this factor with respect of ν is proportional to

$$\theta(1 - \alpha^2) / (1 - \alpha^2)^{1/2}$$

which becomes extremely large as $\alpha^2 \rightarrow 1-0$ [$\alpha = 1$ corresponds to the case where $\omega (= \nu k_z)$ is equal to the angular frequency of the ion-sound waves⁴, $\nu_s(3k_z^2)^{1/2}$]. To introduce a further simplification, I note that the reaction which starts the fusion is the one involving a head-on collision. So, I consider $V(\rho)$ given previously as the modified Coulomb interaction between the nuclei and the velocity ν in it as the relative velocity, ignoring the effects of the centre-of-mass (CM) motion, of two colliding nuclei in the plasma.

With this modified Coulomb repulsion, I obtain, using a standard method⁵, the thermonuclear reaction rate $r = n_1 n_2 \langle \sigma v \rangle$ (where n_1 and n_2 are the number densities of the colliding nuclei) as

$$\langle \sigma v \rangle \simeq \langle \sigma v \rangle_0 |g|^{-1/2} \exp \{ \epsilon_D [\alpha_0 \lambda(\alpha_0) + \theta(1 - \alpha_0^2)(1 - \alpha_0^2)^{1/2}] / k_B T \} \quad (4)$$

where the lowest order in the expansion parameter ϵ_D / E_0 (with $\epsilon_D = Z_1 Z_2 e^2 k_D$) is kept. Here,

$$\langle \sigma v \rangle_0 = 4(2/3\mu)^{1/2} S_0 (E_0^{1/2} / k_B T) \exp(-3E_0 / k_B T)$$

with

$$E_0^{3/2} = (\mu/2)^{1/2} (\pi Z_1 Z_2 e^2 k_B T / \hbar)$$

and

$$\mu^{-1} = M_1^{-1} + M_2^{-1}$$

is the usual reaction rate per pair of nuclei for pure Coulomb repulsion α_0 is $\nu_s(3\mu/2E_0)^{1/2}$ and S_0 is a constant. For a 50% DT mixture, we have $S_0 \simeq 1.3 \times 10^4$ barn keV (in the CM system). The exponential factor in the above equation is, in practice, close to 1 because $(\epsilon_D / k_B T) \ll 1$. So no dramatic enhancement is expected from this factor. The factor g represents a feasible enhancement and it is given by

$$g \simeq 1 - (\epsilon_D / 6E_0) \theta(1 - \alpha^2) [\alpha^2 / (1 - \alpha^2)^{1/2}] \quad (5)$$

where

$$\alpha = \nu_s(3\mu/2E)^{1/2}$$

with

$$E \simeq E_0 \{ 1 - (\epsilon_D / E_0) [(4/3)\alpha \lambda(\alpha) - \theta(1 - \alpha^2)(1 - \alpha^2)^{1/2}] \}$$

In general $g \simeq 1$ except when $\alpha^2 \rightarrow 1 - (\epsilon_D / 6E_0)^2$ or, equivalently, T_e exceeds T such that

$$T_e / T \simeq (2M/3\mu)(3E_0 / k_B T) [1 - (2/3)(\hbar \Omega_p / k_B T)] \quad (6)$$

When the conditions implied in equation (6) are met, $g \rightarrow 0$ which gives the desired anomalous increase in $\langle \sigma v \rangle$. (For a superdense plasma where $T_e \ll T_F$, T_e in equation (6) should be replaced by $2T_F/3$. Here T_F is the Fermi temperature of the degenerate electrons.) For a 50% DT mixture, equation (6) becomes

$$T_e / T \simeq (28/T^{1/3}) [1 - 3.7 \times 10^{-3} (n_e / T)^{1/2}] \quad (7)$$

(The units of n_e and T are 10^{26} cm^{-3} and keV respectively.) For example, for $T = 5 \text{ keV}$, $T_e \simeq 82 \text{ keV}$ is required to satisfy equation (7). Since the bremsstrahlung losses³ are proportional to $T_e^{1/2}$, an additional loss is incurred when $T_e \gg T$ compared with the case when $T_e = T$. And one requires $|g| < (T/T_e)$ (or $|g| < 6.1 \times 10^{-2}$ in the case of a 50% DT mixture) to offset the losses.

In the case when $T_e \ll T$ so that $\nu \gg u_T$ as well as $\nu \gg \nu_T$, we have⁴

$$\epsilon(\mathbf{k}, \omega) = 1 - [\omega_p / (\omega + i\delta)]^2, \quad (8)$$

where $\omega_p = (4\pi n_e e^2 / m)^{1/2}$. In this case, we obtain

$$\langle \sigma v \rangle \simeq \langle \sigma v \rangle_0 [1 + (\hbar \omega_p / 6k_B T)] \times \exp \{ (3E_0 / k_B T) (\hbar \omega_p / 6k_B T) \} \quad (9)$$

Thus, for a superdense plasma, a huge enhancement is expected from the exponential factor. But at such a high density T_F exceeds T , and T_e should be replaced by $2T_F/3$ in μ_T when $T_e \ll T_F$. So in order to have a significant enhancement, we should also require $T \gg T_F$ which gives a limit on n_e as

$$n_e \ll (3\pi^2)^{-1} (2mk_B T / \hbar^2)^{3/2}$$

[or $n_e \ll 1.4 \times 10^{26} T^{3/2} \text{ cm}^{-3}$ (T in keV) for a 50% DT mixture]. I note that even at $n_e = 10^{26} \text{ cm}^{-3}$, $\langle \sigma v \rangle / \langle \sigma v \rangle_0$ is only about 1.2 for a 50% DT mixture at $T = 5 \text{ keV}$. Thus, one finds no significant increase in $\langle \sigma v \rangle$ when $T_e \ll T$.

In conclusion, dynamic screening has a negligible effect in most circumstances, except when T_e exceeds T by the amount

given by equation (6). In this case, an anomalous enhancement in thermonuclear reaction rates occurs

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Garden hose separation of gaseous isotopes

METHODS of separation of isotopes and in particular the separation of uranium isotopes, have long been studied^{1,2}. Here I present a new variation of the "time-of-flight" process, applicable to all gaseous isotopes, although UF_6 gas is considered as the example.

The time-of-flight separation is basically a mechanical analogue of a diffusion barrier. As a cloud of gas moves through an evacuated region, the faster moving lighter molecules concentrate toward the outer portion of the beam configuration, and when they are collected they are enriched in the light component. Similarly, the inner portion would be depleted in the light component when collected. But a continuous and stationary source beam would not give rise to separation as the inner portion of one layer of cloud is equivalent to the outer portion of the subsequent layer of cloud. A 'chopper' is therefore required to cut the beam into individual layers, and extensive pumping is needed to evacuate the system sufficiently to maintain the integrity of the cloud during their time of flight.

During World War II, Bagge designed such an apparatus³, with synchronised choppers, collectors, and powerful pumps, a cascade of such time-of-flight units were constructed to separate the uranium isotope. But the necessity of operating that system at very low gas pressure and with limited efficiency of collection are major drawbacks implying low throughput and high consumption of pumping energy.

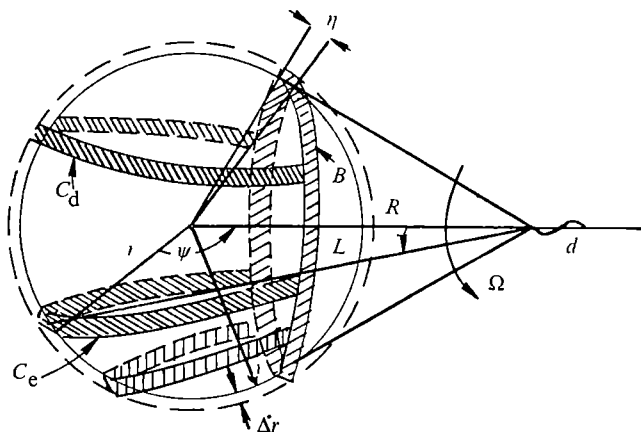


Fig. 1 Schematic relationship between the population fronts and the collectors B, C_e, and C_d, where η is the angular span of collector B. B and C_e are for enriched light component, while C_d is for depleted light component. Δr is the mean separation of the population fronts.

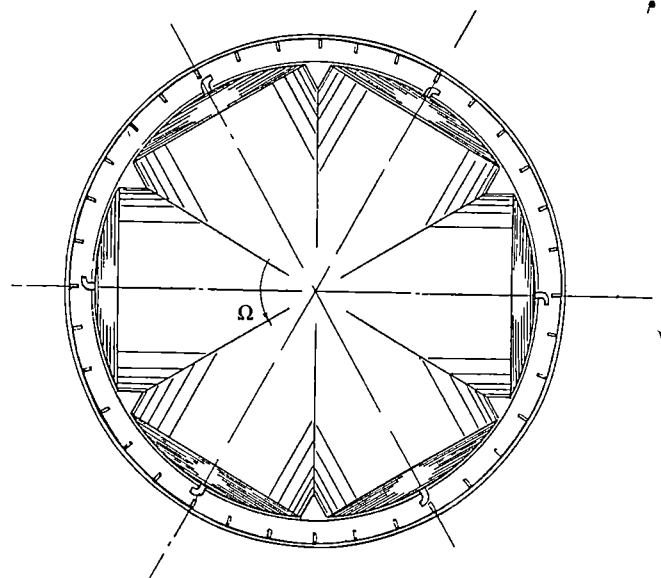


Fig. 2 A diagrammatic plan view of the centrifugal separating chamber with three circular compartments at the wall storing the enriched, depleted, and to-be-recycled concentrations. For $v_e \approx 2v_0$, there are six ejection outlets symmetrically disposed in a plane of rotation, with each outlet standing for three separate outlets stacking on top of each other for their corresponding storage chambers.

In the technique I propose, the gas molecules are channelled into an evacuated chamber through a set of rotating supersonic nozzles at a small retarding angle so that the ejected gas has zero angular momentum. Once inside the vacuum chamber, molecules travel according to their corresponding thermal velocities plus a common ejection velocity v_e , and from

$$mv_0^2/2 = 4kT/\pi = \text{constant} \quad (1)$$

the difference in mass gives,

$$\Delta v_0 = -\Delta mv_0/2m = -(2kT/\pi m)^{1/2} \Delta m/m \quad (2)$$

From the Maxwellian equal partition relationship, we have $\Delta v/v = -\Delta m/2m$ in general. After time t , while the ejection velocity carries the molecules to a distance $R = v_e t$ from the nozzle, the thermal velocity would carry them into spherical fronts of radius r centred at the end point of R , and the separation of the fronts Δr is

$$\Delta r = \Delta vt = -\Delta mvR/2mv_e = 0.426\% \text{ of } vR/v_e \quad (3)$$

for UF_6 .

The molecular fronts are now in the form of a ball (thermal velocities) on top of a cone (ejecting velocity), so the objective is to design rotating collectors to skim the surface of the ball most effectively in order to collect a substantial portion of the concentrations separated by the two sides of the ball surfaces. Figure 1 is a schematic of collectors C_e (for the enriched front), C_d (for the depleted front) and B (for the relatively stationary collector of the original Becker design), the angular width of collector B can be shown as $\approx 2 \cos^{-1} (1 - \Delta r/r) \approx 10^\circ$.

Once the molecule enters the collector, it would be swung toward the chamber wall by the centrifugal force, and it is stored and pressurised by the rotating wall outside the chamber. For $v_e = 2v_0$, there can be as many as six sets of nozzle-collector combinations operating at the same plane (Fig. 2). The storage chamber has three compartments for enriched, depleted, and to-be-recycled concentrations. The last part is

mainly the 'unskimmed' portion which is channelled back to the original nozzles that ejected them. There are no moving parts relative to the chamber in this collector-nozzle-chamber combination, and by operating under vacuum (~ 0.1 torr), UF_6 can be stored in its solid form so that the flow can be controlled by thermal evaporation as well.

Following the Maxwellian distribution, the normalised population density $n(v)$ is,

$$n(v) = (m/2\pi kT)^{3/2} 4\pi v^2 \exp(-mv^2/2kT) \quad (4)$$

where

$$\int_0^\infty n(v) dv = 1$$

and

$$\int_0^\infty n(v) v dv = (8kT/\pi m)^{1/2}$$

Consider first the high energy Maxwellian tail, for $v' \geq v_0$, let $v^2 = v'^2 + u$, $2kT/m$ and $g \equiv mv'^2/2kT$, so the fractional population at the tail portion is

$$\begin{aligned} n(v \geq v') &= \int_{v'}^\infty n(v) dv = (2/\sqrt{\pi}) e^{-g} \int_0^\infty (u+g)^{1/2} e^{-u} du \\ &= (2/\sqrt{\pi}) e^{-g} [g^{1/2} + 1/2 g^{-1/2} - 1/4 g^{-3/2} + \dots] \end{aligned} \quad (5)$$

and calculate the increment Δn by differentiations of $n(v)$ and integrate,

$$\Delta n(v \geq v') = (-4/\sqrt{\pi}) e^{-g} g^{3/2} \Delta v'/v' \quad (6)$$

then

$$\Delta n/n \simeq [-8g^3/(4g^2+2g-1)] \Delta v'/v' \quad (7)$$

Similarly, at the low energy part of the spectrum where $g \ll 1$,

$$n(v \leq v') \simeq (4/\sqrt{\pi}) g^{3/2} (1/3 - g/5 + g^2/14) \quad (8)$$

and

$$\Delta n/n \simeq e^{-g} (\Delta v'/v') / (1/3 - g/5 + g^2/14) \quad (9)$$

Figure 3 shows plots of equations (5)–(9). Clearly, concentrating on the high energy tail alone, α can be made as large as that of total separation, only at the expense of reducing the total fractional population by the Boltzmann factor e^{-g} . The depleted fractional from equation (9) has a relatively stable separation at $\Delta n/n \simeq 1\%$.

I now make a simple estimate of the specific power requirement of the 'gross-cut-mode' from equations (7) and (9). Let y be the enriched stream, x the depleted stream, θ the cut of separation, then $y = \theta(1 + \Delta n/n)$, $x = (1 - \theta)(1 - \Delta n/n)$, and from $\alpha \equiv (y/(1-y))/(x/(1-x))$, ignoring terms with $(\Delta n/n)^2$, we have

$$\alpha \simeq \theta(1 + \Delta n/n) / [(1 - \theta)(1 - \Delta n/n)] \quad (10)$$

Choose $|\Delta n/n| \simeq 1\%$ for both x and y , so from Fig 3,

$$n(v \geq v' \text{ at } g \simeq 2) \simeq 25\% \simeq n(v \leq v' \text{ at } g \simeq 0.7)$$

and let the collectors catch one-half of the designated fractionals, so one-quarter of the population is processed while

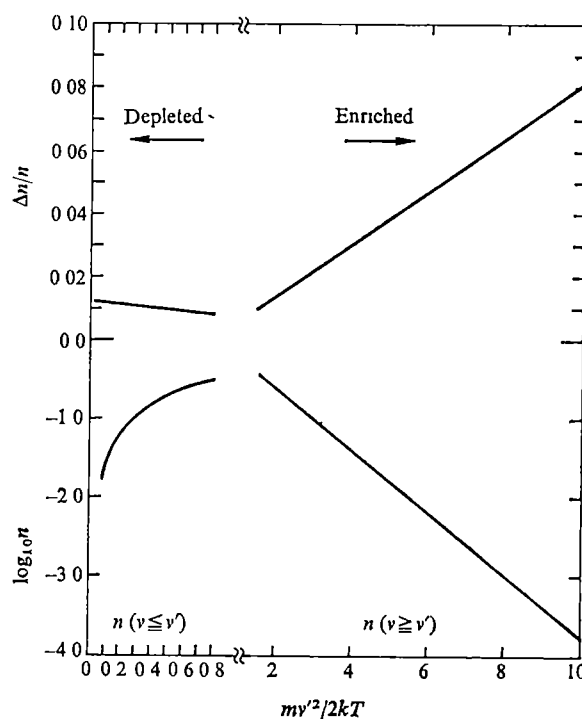


Fig. 3 Concentrating on the high energy tail of the Maxwellian distribution, $\alpha \simeq 1 + 4\Delta n/n$ can be made large at the expense of reducing fractional population collected. At the low energy end, $\Delta n/n$ is relatively stable at 1%. A crude estimate of processing a quarter of the population at each end of the spectrum gives a specific power consumption only one-fifth that of the minimum theoretical diffusion requirement.

three-quarters is channelled back to the nozzles to be recycled. With $\theta = 1/2$, from equation (10),

$$\alpha \simeq 1 + 4\Delta n/n \simeq 1.04 \quad (11)$$

Let $R = 100$ cm, $r = 50$ cm, and the height of the chamber $D \simeq R + r = 150$ cm, $\Omega = 2\pi \times 30$ Hz so the velocity of the collector $v_c \simeq \Omega R \simeq 2v_0$ at room temperature. The mean free path $\lambda \simeq D \simeq 150$ cm so the pressure $P_0 = n_0 kT \simeq 1.75 \times 10^{-2}$ dyne cm^{-2} with molecular diameter $\simeq 6$ Å. Because of the centrifugal pumping, once the molecules are caught by the collectors, the gas pressure is raised by the factor $mv_c^2/2kT = 5.8$ so pressure in the collector $P_c \simeq 0.1$ dyne cm^{-2} . The number of molecules N flowing through the processing chamber per second is

$$\begin{aligned} N &= 6 \times v_c \times n_0 \times \pi(D/2)^2 \\ &= 1.2 \times 10^{21} \\ &= 0.7 \text{ g of } \text{UF}_6 \\ &= 15 \text{ t of uranium per year} \end{aligned} \quad (12)$$

The separating collectors carry only a quarter of this value, so the throughput is $15 \times 1/4 = 3.75$ t yr^{-1} . The power requirement for the collectors and the shields acting as a centrifugal pump is

$$0.5 mv_c^2 \text{ s}^{-1} = 28 \text{ W} \quad (13)$$

By balancing the compression energy of the gas with that of the jet flow of $0.5 mv_c^2$ at 25 W, the nozzle opening should be about 0.5 cm and from the continuity of flow, $P_{\text{nozzle}} = P_0 \times (150/0.5)^2 = 9 \times 10^4 P_0$, so the additional compressional power required is

$$(m/M) RT \log(P_{\text{nozzle}}/5.8 P_0) \simeq 20.7 \text{ W}, \quad (14)$$

where R is the gas constant, M the molecular weight of UF_6 and

\dot{m} the mass flow in g s^{-1} . The Unit-of-Separative-Work (USW) done for each stage is now,

$$\begin{aligned}\text{USW} &= \text{Throughput} \times (\alpha - 1)^2 / 2 \\ &= 3.75 \times 10^3 \text{ kg yr}^{-1} \times 0.04^2 / 2 \\ &= 3.0 \text{ kg yr}^{-1}\end{aligned}\quad (15)$$

and the specific power requirement is,

$$\frac{1}{2} (0.028 + 0.0207) = 0.0162 \text{ kW}/(\text{kg USW}) \text{ yr}^{-1}, \quad (16)$$

which is only 22% of the theoretical minimum requirement of the diffusion process ($0.073 \text{ kW}/(\text{kg USW}) \text{ yr}^{-1}$). The throughput of this method is relatively low so the specific capital cost may be higher. On the other hand, by using UF_6 in a solid form for storage, together with a much higher separation coefficient, the number of processing stages required can now be reduced by over an order of magnitude. The present method also compares favourably with a centrifuge in terms of centrifugal force requirement and the choice of variable separation constant α .

Some of the recent methods of multistage preferential heating of isotopes by lasers can also be incorporated into the present method, as the time-of-flight vacuum chamber preserves the original state of motion as well as that added and the chamber dimension near the axis of rotation is such that it can easily accommodate these additional arrangements.

I thank Dr M. Benedict of MIT for showing me related literature, my colleagues in Combustion Engineering for comments and Mr A. Davis for drawing Fig. 2.

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Radium removal from drinking water

RADIUM is absorbed from natural waters on to acrylic fibres impregnated with oxides of manganese¹. This extraction technique, developed for oceanographic studies, is also effective in removing radium from drinking water.

Radium-226, a naturally occurring radioactive daughter of ^{238}U , presents a potential health hazard when dissolved in ground waters. The body metabolises radium and calcium similarly, if radium is available to the bloodstream, it will concentrate in the bones². Radioactive decay of ^{226}Ra within the bones exposes the skeleton to highly ionising, short range alpha particles as well as more penetrating beta and gamma radiation from radium daughters. The most immediate effect of this radiation is to increase the chances of developing osteosarcoma and other cancers². Although the exact relationship between the low level radium exposure and cancer risk is not known, there is probably no safe level of human exposure to radium. The drinking water standards published by the United States Public Health Service recommend³ a level not exceeding 3 pCi l^{-1} ($6.66 \text{ d p m l}^{-1}$).

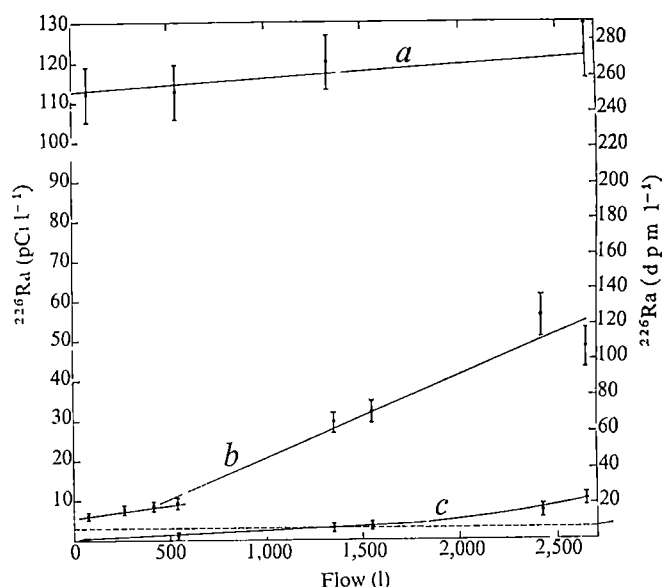


Fig. 1 Concentrations of ^{226}Ra measured in the water from the well and in the effluents from two columns filled with manganese-fibre. The first 500 l were processed at 4.3 to 6.2 l min^{-1} , the remainder at 7.5 to 8.2 l min^{-1} . The variable slopes of the first column effluent reflect this difference in flow rate. a, Well effluent, b, first column effluent, c, second column effluent. ---, US Public Health Service drinking water limit.

Commercial grade uranium deposits in south Texas have been mined since the late 1950s. In 1971, elevated ^{226}Ra levels were measured in well water near the edge of the Felder uranium ore body near Three Rivers, Texas⁴. Other wells in the south Texas area have been surveyed and a small fraction produce drinking water with radium levels above the United States Public Health Service Standard⁵. One of these wells, referred to as Whitley well No. 1, is located about 8 km south-west of George West, Texas. This well has a ^{226}Ra concentration of 110 pCi l^{-1} , the second highest level measured in recent surveys of this area⁵. Small deposits of uranium occur within 300 m of this well, a high grade uranium deposit is being mined within 8 km. These localised ore bodies are believed to be the sources of radium found in the well waters of south Texas. Wells known to produce water high in ^{226}Ra have been removed from use as routine drinking water sources.

Acrylic fibre impregnated with manganese has been used to remove radium from seawater. We prepared similar fibre using the procedure described by Moore and Reid¹. The resulting fibre contained 12–15% Mn by weight when dry.

Two water filtering columns connected in series were each filled with 40 g of Mn fibre. The columns were attached to Whitley well No. 1. A water meter attached downstream of the columns recorded the volume of water processed. Water samples were periodically drawn (a) directly from the well, (b) from the column 1 effluent, and (c) from the column 2 effluent. These samples were stored for ^{226}Ra determinations using the ^{222}Ra emanation technique⁶.

Initially the flow rate decreased slightly due to packing of the Mn fibre. By adjusting the water pressure we attained a steady flow of 7.5 to 8.2 l min^{-1} after about 550 l had passed through the system. The experiment was terminated after 2,650 l had been processed.

Our objective was to determine if radium could be effectively and inexpensively removed from this well water. Figure 1 shows that radium removal from this highly contaminated well by two Mn-fibre columns was essentially complete for 1,300 l. After 1,300 l had passed through the system, the column 2 effluent was 3 pCi l^{-1} , the Public

Health Service recommended drinking water limit. After 2,650 l of water had been processed, the outflow from column 2 still had less than 10% of the ^{226}Ra concentration of water entering the system. We conclude that radium removal from highly contaminated ground waters is technically feasible using manganese impregnated fibres. Although this experiment represents only one well, our success with this water as well as similar success with seawater and brackish water which contain high concentrations of calcium and magnesium¹, makes us optimistic about the general applicability of the Mn-fibre technique for removing radium from drinking waters.

The cost of removing radium using the Mn fibres is low. The fibre can be prepared for about \$15.00 per kg. One kilogramme fibre would bring the radium level of more than 10,000 l of the water from Whitley well No 1 to within the United States Public Health Service limit. This would be adequate drinking water for a family of four for 3 yr (ref. 7). Since most wells in the area have ^{226}Ra levels much below that of Whitley well No 1, the 10,000 l figure is a minimum. We conclude that manganese fibres can provide an inexpensive means of removing radium from drinking water.

We emphasise that this work is still in the testing stage. Any attempts to decontaminate drinking waters with respect to radium should be conducted only under the supervision of local health authorities.

We thank Mr and Mrs Sonny Whitley for the use of their well. Encouragement from Dr H. C. Eppert, jun and Dr Morris Schulkin of NAVOCEANO and Mr Martin Wukasch, P.E., Director, Division of Occupational Health and Radiation Control of the Texas State Health Department is acknowledged.

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Methylation of lead in the environment

LEAD is one of the most toxic metals found in the environment and is of great concern because of its widespread occurrence in nature, but its fate is largely unknown¹. Much of the lead dispersed by man is eventually washed into natural waters and is presumably precipitated into the sediments². The methylation of mercury and arsenic by microorganisms in the environment has been documented and well summarised³. Nothing, however, has been known about the existence of organic forms of lead in the environment as a result of biotransformation⁴.

Here we present evidence for the first time, that microorganisms in lake sediments can transform certain inorganic and organic lead compounds into a volatile tetramethyl lead (Me_4Pb). Experiments were carried out with 50 g of sediment and 150 ml of lake water from Hamilton harbour (Lake Ontario), Mitchell Bay (Lake St. Clair), and Erieau harbour (Lake Erie), placed individually in a

250 ml filtering flask fitted with a side-arm. Nutrient broth (0.5%) and glucose (0.1%) were added to stimulate the microbial growth and the flask was capped and sealed. Nitrogen was bubbled in to create an anaerobic condition but this practice was later discontinued because the microbial growth was sufficient to generate the anaerobic condition. After incubation for 2 weeks at 20°C, the gas phase in the incubation flasks was withdrawn through the side-arm by means of a peristaltic pump and transferred to a U-tube containing 3% OV-1 at -70°C. The sample trapped in the U-tube was swept into a gas chromatograph-atomic absorption spectrophotometer system for the separation and analysis of the volatile lead compound. Tetramethyl lead was detected in the air sample with reference to the retention time of a synthetic compound. The identity of the Me_4Pb peak in the air sample was further confirmed by gas chromatography and mass spectrometry. Addition of inorganic lead nitrate or organic trimethyl lead acetate (Me_3PbOAc) at 5 mg (expressed as lead) per litre of sample greatly increased the Me_4Pb production. Not all sediment samples we examined, however, produced Me_4Pb in identical conditions to those described above. In some cases, the addition of inorganic lead nitrate or chloride failed to affect the transformation but in all cases the transformation of Me_3PbOAc to Me_4Pb was observed. There was no transformation if the systems were autoclaved. Ultraviolet light irradiation caused no conversion of Me_3PbOAc to Me_4Pb in the absence of microorganisms, so ruling out the possibility of chemical disproportionation reactions⁵, activated by ultraviolet light.

Direct chemical synthesis of methyl lead compounds through alkylation of inorganic lead salts is very difficult because of the extreme instability of the postulated first intermediate monomethyl lead salts. We have observed the conversion of lead nitrate and lead chloride to Me_4Pb on several occasions in lake sediments but no Me_4Pb transformations have yet been detected from lead hydroxide, lead cyanide, lead oxide, lead bromide, or lead palmitate. In none of these experiments were Me_3Pb^+ salts detected in the culture medium, although it is a stable intermediate in the chemical pathway of the successive methylation. (The analysis for trimethyl lead salts was specially developed in our laboratories in support of this study.)

The pathways for the biological conversion of lead are not well understood. It is apparent that the conversion of inorganic lead to organic lead is a difficult process and it probably requires specific physical, chemical, as well as biological conditions. On the other hand, the biological methylation from Me_3Pb^+ to Me_4Pb seems to proceed quite readily and we were able to demonstrate this conversion with pure species of bacterial isolates from Lake Ontario without the presence of sediment. We found that species of *Pseudomonas*, *Alcaligenes*, *Acinetobacter*, *Flavobacterium*, and *Aeromonas* growing in the presence of nutrient broth (0.5%), glucose (0.1%) and yeast extract (0.1%) could transform Me_3PbOAc into Me_4Pb . None of the isolates was able to produce Me_4Pb from inorganic lead in chemically defined media.

Weekly sampling of the atmosphere in the incubation flasks was carried out to determine the rate of conversion of Me_3PbOAc to Me_4Pb . With 10 mg of lead (as Me_3PbOAc) in the sample, 125 µg of lead (as Me_4Pb) was detected after incubation for 1 week. The conversion rate increased to 642 µg in the second week and declined to 550 µg and 256 µg of lead in the third and fourth weeks respectively. The decrease in lead conversion after 2 weeks could be due to the exhaustion of nutrients, accumulation of toxic metabolic products, or changes in pH in the medium. Assuming that 642 µg of lead is the maximum rate for the conversion, the microorganisms were able to convert 6% of lead in one week.

The toxicity of Me_4Pb towards algae was studied by

bubbling the biologically generated Me_4Pb from one flask (containing 5 mg Pb l^{-1} as Me_3PbOAc) into the culture medium in another flask where a test alga *Scenedesmus quadricauda* was grown. As Me_4Pb is not soluble in water and is volatile, the exposure of an alga to this lead compound was only momentary. We estimated that less than $0.5 \text{ mg Pb}(\text{Me}_4\text{Pb})$ had passed through the culture medium. We found that the primary productivity and cell growth (determined by dry weight), however, decreased by 85% and 32% respectively, as compared with the controls without exposure to Me_4Pb . Furthermore, cells exposed to Me_4Pb tended to clump together. Similar results were obtained with *Ankistrodesmus falcatus*. To obtain similar inhibition, twice as much lead, in the form of Me_3PbOAc , and twenty times as much lead nitrate would be required. These numbers were calculated from our studies on the toxicity of various forms of lead compounds on algae.

We conclude from the results of 50 experiments that incubation of some lead containing sediments generates Me_4Pb , that Me_3Pb^+ salts are readily converted to Me_4Pb by microorganisms in lake water or nutrient medium, with or without the sediment, and in the presence or the absence of light, that conversion of inorganic lead (such as lead nitrate or lead chloride) to Me_4Pb occurred on several occasions in the presence of certain sediments, and that the conversion is purely a biological process.

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A study of age group track and field records to relate age and running speed

AGE affects man's ability to run fast but to date there has been little interest in quantitating the relationship. The few published studies, such as the one by Dill on marathon runner Clarence DeMar¹, are concerned mainly with reporting lung volume, heart rate, and other physiological characteristics of notable performers, with only brief reviews of race performances over the runner's life span.

Two recent compilations of track and field records present an opportunity to study ageing effects on running speed. One contains records for men², in 1-yr age groups for ages 1 to 78, and the other contains women's records³, in 1-yr age groups for ages 3 to 60. This study was designed to determine how rapidly running speed deteriorates with age and whether the rate of deterioration depends on the length of the race, and to compare deterioration rates of running speed, strength and stamina in men and women.

When speed (m s^{-1}) is plotted against age, it is evident that speed improves up to age 20 or so and gradually deteriorates beyond age 30. Between ages 20 and 30, running speed is near the maximum and almost constant for all distances included in the records (100 m, 200 m, 400 m, 800 m, 1,500 m, 3,000 m, 5,000 m, 10,000 m and marathon (42,195 m)). An exponential model was chosen to fit the age records for each distance.

$$Y = A_1 [1 - \exp(A_2 X)] + A_3 [1 - \exp(A_4 X)],$$

where Y is the speed in m s^{-1} , X is the age in years, and the constants A_1 , A_2 , A_3 , A_4 are determined from the age records by the method of least-squares. This model has the advantage of fitting the data well over the entire age range, whereas the more usual Gompertz-type equations can be applied only to ages where speed is decreasing with age. In addition, this model allows the rate of increase in speed (under age 20) to differ from the rate of decrease (over age 30). The model also goes through the origin so that at zero age the speed is zero. Examples of the fit for several running events are shown in Fig. 1.

A comparison of the fit curves for all the men's running events reveals that age of maximum performance increases with distance. This is also true of the recorded data, where the sprint records are held by men in their early twenties but the marathon record-holder is 26 yr old. Comparison of the slopes of the fit curves reveals that speed deteriorates more slowly at longer distances than at shorter ones. For example, at age 50 speed in the 200 m sprint is slowing at a rate of $0.09 \text{ m s}^{-1} \text{ yr}^{-1}$ but for the marathon the rate is only $0.06 \text{ m s}^{-1} \text{ yr}^{-1}$. This observation suggests that strength deteriorates faster than stamina.

This hypothesis was tested further by fitting the exponential model to the age records for the men's shot-put and discus, events which are even more strength-dependent than the 200 m sprint. Direct comparison of the slopes for running events with those for the field events is not valid because the recording units differ. A comparison can, however, be made if all age records are converted to percentages of the world record.

Fig. 1 Running speed records by age. Exponential curves (for model, see text) were fitted to published age records: (a) Men's records for 200 m, 800 m, and marathon (42,195 m); (b) The 400 m records for men and women.

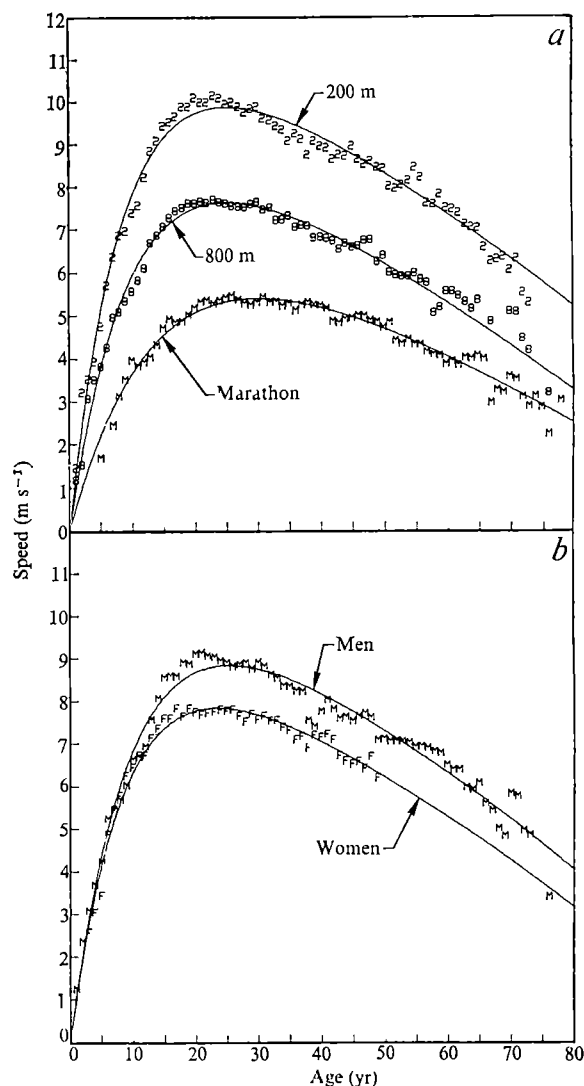


Table 1 Age records as percentages of world records

Age	Shot-put	Discus	200 m	Marathon
10	61	60	79	68
20	91	90	98	94
30	100	100	99	100
40	98	95	93	96
50	76	78	84	88
60	49	53	74	76

Percentages based on fitting the exponential model (see text), to published data

Thus, a speed of 8.12 m s^{-1} for the 200 m race is 80% of the world record speed (10.15 m s^{-1}), whereas a shot-put of 17.46 m is 80% of the corresponding world record (21.82 m). These comparisons (Table 1) lend further support to the hypothesis that strength deteriorates faster with age than does stamina.

Fewer data are available on women's age records (current records only include running events up to 3,000 m, and rarely are there records for women over 45 yr of age). A comparison with those for men suggests that girls mature faster than boys (with respect to running events) in the age range 8–12 yr. For example, at ages 8, 9 and 11 women's records are faster than those for males in the 400 m run (see Fig. 1b). It also appears that beyond age 30, speed deteriorates at a faster rate for women than for men. For example, the fit curves show that in the 100 m sprint the women's record at age 45 is 85% of the women's world record, whereas the corresponding figure for men is 90% of the men's world record.

The records analysed here comprise the marks of many individual athletes but they can be thought of as those set by a 'super' runner, one who is in top condition throughout his life span. The ordinary runner is one whose speed is slower, perhaps by some fixed amount over the entire age span, than that of the super runner. The speed curve for the ordinary runner may have the same shape as that of the super runner and the rates of change of speed with age would be the same. This hypothesis could be tested by following up several runners over their life spans, recording annually their running times over measured distances. This could be done retrospectively, by contacting those who have run for a number of years and determining their best times at various distances each year.

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The capacity for joint visual attention in the infant

LITTLE is known about how visual attention of the mother-infant pair is directed jointly to objects and events in the visual surround during the first year of the child's life. To what extent does the child follow the mother's lead and the mother the child's, and what are the processes involved? The ability of the infant to respond successfully to such signals allows the mother to isolate and highlight a much wider range of environmental features than if the infant ignores her attention-directing efforts. We report a preliminary investigation of the extent of the infant's ability to follow changes in adult gaze direction during the first year of life.

Mothers who had volunteered as subjects in response to

newspaper advertisements were asked to bring their infants into our laboratory at a time when the infant was 'usually active'. Thirty-four infants, 2–14 months old, were tested in a small, sparsely-furnished room (15 ft by 10 ft) with a one-way screen at one end and a window with drawn blinds at the other. The infant was placed in a highchair, appropriate to his age, in an upright position. The mother played with him until he seemed settled and was then replaced by the experimenter—either male or female in their twenties, unknown to the baby. It was not practicable to have the mothers as experimenters with the rather strict testing requirements used here although many observations were made prior to this on other mothers and infants in a similar, though less controlled, situation. The experimenter first played with the infant for a short period and, if the latter showed no signs of distress, the mother left the room. The experimenter remained seated in front of the infant, eyes at the same level, about 0.5 m away. The infant was then given two trials in a prearranged order.

On each trial the experimenter first made eye-to-eye contact then silently turned his (her) head through 90° to fixate a small (concealed) signal light, 1.5 m away, for 7 s. He then turned back to interact with the infant. Two trials were given, one involving a head turn to the right, one a turn to the left, with inter-trial intervals varying from 20 to 50 s, depending on difficulties in establishing subsequent eye-to-eye contact. The infant's behaviour was recorded by two concealed video cameras set at 45° to the experimenter-infant face-to-face axis, giving a split-screen 45° profile of the infant from two sides. The experimenter was not visible on the screen. The infants were also filmed for calibration looking at experimenter in fixed positions of known angular displacement.

Trials were scored from the videorecord by the experimenter, based on infant head movement only. A positive response was scored if the infant looked (a) in the same direction (right/left) but not down at the floor or up at the ceiling, (b) without an intervening look elsewhere (ignoring short looks down during postural adjustment), (c) within 7 s, and (d) appeared to be looking for or at something (involving halting the head turn for 0.5 s or more with a cessation of limb movement). The infant did not have to appear to be fixating exactly the same point. Scoring reliability was ascertained from three naive observers uninformed as to experimenter's judgment. They produced 96% agreement on trials scored as positive, 90% on those scored as negative.

The proportion of infants judged as having produced a positive response on one or both trials increases steadily with age (Table 1). The form of the response, that is, how soon and where the infants look, did not show systematic change with age. Latencies in response were very variable—any point up to the end of the trial, and the infants would look anywhere from about 20° to 90° away from the midline. Labelling responses as positive does not, of course, mean that infants were looking for something to look at. In the upper age groups, however, there was strong evidence to suggest this may be so with infants often looking away, looking back at the experimenter and then looking away again. This had been even more marked when the mother was the experimenter in earlier observations. Of the negative trials over 80% were comprised of responses where the infant either kept his eyes on the experimenter or looked down at the highchair table top.

The proportion successfully responding may well be depressed by the strangeness of the setting although the incidence

Table 1 Percentage of children judged as following line of regard in one or both trials

Age (months)	No. infants	% Showing positive response
2–4	10	30
5–7	13	38.5
8–10	6	66.5
11–14	5	100

of following line of regard is enhanced by pointing and exclamatory vocalisations such as "Oh look" By 11 months, however, some children gave unequivocal positive responses to eye movement alone Some (from 6 months) even followed line of regard when the experimenter and mother, not interacting with the infant, conversed a metre away from the child

It is possible that the ability to orient with respect to another has implications for Piaget's¹ more complex notions of the egocentric child In so far as mutual orientation implies a degree of knowledge in some form about another person's perspective then the child in its first year may be considered as less than completely egocentric The source of such abilities (for example, imitation) remains to be investigated but utilisation of another's gaze direction may be a very basic process Cooke's Hartebeeste (*Alcelaphus buselaphus*) on hearing a danger signal raise their heads, look at the signaller and orient in the direction he is facing (M Stanley-Price, personal communication) Such observations need careful investigation but it may not be entirely unexpected that human infants should also have greater abilities than has been supposed (for example, Schaffer²).

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Role of juvenile hormone esterases and carrier proteins in insect development

THE discovery of a binding protein, specific for juvenile hormone (JH)¹ in the haemolymph of the tobacco hornworm, *Manduca sexta*, suggested that this protein carries JH molecules from the secretory organs (corpora allata) to the target sites We report here that the binding protein provides the hormone total protection from degradative enzymes present in the haemolymph throughout early larval life^{2,3} Just before the start of pupal differentiation, however, a new esterase appears in the haemolymph which is able to hydrolyse the protein-bound hormone We have also found that the binding protein at concentrations of 10^{-8} M strongly enhances the development-inhibiting action of JH on wing disk tissue *in vitro* These properties, taken together, demonstrate a carrier role for the binding protein, and give new insights into the control of hormone levels in the haemolymph

Two closely related forms of the binding protein (CP-1 and CP-2) were purified to homogeneity by gel filtration on Sephadex G-100 (0.005 M Tris-HCl, pH 7.3, 0.1 M NaCl), followed by ion-exchange chromatography on Biogel DEAE (0.005 M Tris-HCl, pH 8.3, 0.1–0.14 M NaCl gradient) and preparative isoelectric focusing (pH 4–6, 1% Ampholine, 500 V, 72 h) The binding proteins elute as a single peak from the Sephadex column but are separated on the ion-exchange column and are finally purified by isoelectric focusing Under these experimental conditions CP-1 ($pI = 4.8$) was the major binding protein (>75% of the total hormone binding capacity) and was used for subsequent studies The hormone-protein complex formed with pure CP-1 and that formed in crude haemolymph showed that same dissociation constant ($K_D \approx 10^{-7}$ M)¹ and slow dissociation rate ($t_{1/2} = 15$ min)

The JH hydrolytic activity in the haemolymph of fifth instar larvae can be separated into two distinct peaks by gel filtration on Sephadex G-100 (0.005 M Tris-HCl, pH 7.3, 0.1 M NaCl) The activity in peak I (molecular weight $\approx 10^6$) is totally inhibited by 10^{-4} M diisopropylphosphorofluoridate

Table 1 Carrier protein protection of JH against hydrolysis by esterase fractions isolated from haemolymph of fifth instar *M sexta* larvae

Esterase fraction	Hydrolysis of JH (%)	Protection (%)
	–CP* +CP†	
Peak I	43 0	100
JH-esterase A	74 70	5

Pure synthetic *Hyalophora cecropia* JH¹ (methyl *trans,trans,cis* 3, 11-dimethyl-7-ethyl-10,11-epoxytrideca-2,6-dienoate) labelled in the chain (7-ethyl-1,2-³H, 67 mCi mg⁻¹ (New England Nuclear Corp) was used Reaction products were separated and identified by thin-layer chromatography according to the method of Slade and Zibitt² and quantitated by liquid scintillation counting

*Incubation for 15 min at room temperature of 20 μ l esterase and 10 μ l JH (8×10^{-7} M) followed by addition of 10 μ l carrier protein (4×10^{-5} M), incubation for 2 min and then addition of 10 μ l charcoal (4.5 μ g) to remove unbound JH Charcoal removed by centrifugation at 8,000g for 2 min Supernatant applied immediately to thin-layer chromatographic plate for analyses

†Same procedure as described for –CP but CP and JH were pre-incubated for 2 min before addition of esterase

(DFP) (pH 7.0, 15 min) whereas that in peak II (molecular weight $\approx 6 \times 10^4$) is unaffected under the same conditions Using this concentration of DFP, the crude haemolymph can be assayed for the two types of esterases Enzyme class II has been further purified by preparative isoelectric focusing (pH 4–6, 1% Ampholine, 500 V, 72 h) and three closely related forms (JH-esterases A, B and C) were separated All three forms show equal rates of hydrolysis of JH, and JH-esterase A was routinely used for our experiments

The hydrolysis of the hormone by JH-esterase A was measured in the presence and absence of binding protein CP-1 Table 1 shows that none of the enzymes of family I can hydrolyse JH complexed to CP-1 On the other hand, JH-esterase A hydrolyses the hormone even in the presence of a large excess of CP-1 In an attempt to correlate the presence of JH-esterases with the levels of JH in the insect, we examined the haemolymph from fourth instar larvae and found that esterases of type II are virtually absent In view of these results it was of interest to study the level of type II esterases during the critical premetamorphic period of the fifth instar Figure 1 shows that the level of the JH-esterases increases considerably and reaches a peak on day 5 of the fifth instar while type I esterases show relatively little variation during the same period

These results suggest important functions of both the binding protein and JH-esterases in the regulation of the JH level in the haemolymph during larval development In the early instars, where high levels of JH are required, the binding protein is necessary to protect JH from degradation by type I esterases Because of the continuous presence of these esterases, only JH complexed to the binding protein can reach the target tissues and thus the binding protein is the true carrier in the haemolymph In the fifth instar, on the other hand, type II esterases could constitute the most important factor in lowering the JH level in the haemolymph, thus permitting metamorphosis

To test further the participation of the carrier protein in the inhibitory action of JH in insect development, we used the technique of Oberlander and Tomblin⁴ This method measures the action of JH in terms of the inhibition of ecdysone-induced cuticle deposition in tissue cultures of imaginal wing disks from the Indian meal moth, *Plodia interpunctella* Table 2 shows the considerable synergistic action exerted by low levels ($< 10^{-8}$ M) of *M sexta* carrier protein on JH inhibition of *Plodia* wing disk metamorphosis The protein alone has no effect in this system Thus the carrier protein acts not only in haemolymph during transport of the hormone but also at the target issue Because of the high level of hormone and the very low level of carrier protein (well below K_D) required to evoke a large synergistic effect, it is unlikely that this is simply a protective effect of the carrier against degradative enzyme, which is known to occur in disk tissue⁵ Rather, the

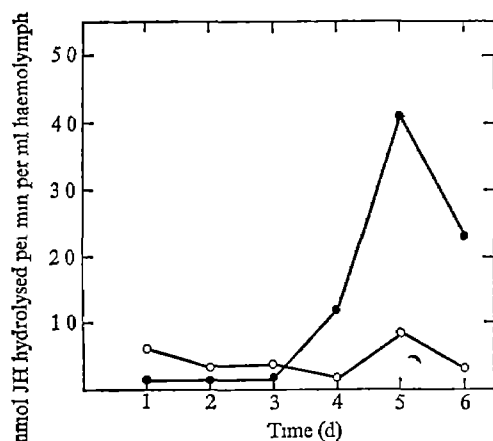


Fig. 1 Activity of general and JH-esterases in the haemolymph of fifth instar *M sexta* larvae of different ages. Haemolymph was collected as previously described¹ and kept at -20°C until used. Activities were assayed using a newly developed charcoal assay method and specially prepared *M sexta* JH (methyl *trans, trans, cis* 3,7,11-trimethyl-10,11-epoxydodeca-2,6-dienoate) labelled in the ester methyl group (methyl- ^3H , 8.3 mCi/mmol⁻¹), (L L S, K J K, F J K, and J H L, unpublished). The reaction mixture was composed of 50 μl of JH solution ($4.45 \times 10^{-5}\text{ M}$) in 0.1 M phosphate buffer, pH 7.0, 10 μl of 10% methanol and 50 μl of haemolymph in a 5 ml centrifuge tube. The reaction was quenched with 10 μl glacial acetic acid after which 100 μl charcoal suspension (0.045 mg) was added. The sample was mixed thoroughly and allowed to stand for 20 min and then centrifuged at 15,000g to sediment the charcoal. An aliquot of the supernatant was removed and the amount of radioactive methanol liberated was determined by liquid scintillation counting. DFP-inhibited samples (●) represent JH-esterase activity while non-DFP-inhibited samples represent total JH hydrolytic activity in the haemolymph. General esterase activity toward JH (○) is the difference between JH-esterase and total JH hydrolytic activities. Gut purging preparative to metamorphosis occurs on day 7 in our *M sexta* colony.

carrier probably serves a function in hormone recognition or transport at the target tissue. High molecular weight lipoproteins have been suggested as JH carriers in saturniid moth pupae and adults⁶ and in adult locusts⁷. In the *Plodia* tissue culture system, preliminary results indicate that the lipoproteins from *Hyalophora cecropia* and *P interpunctella* had no observable synergistic effect.

By their ability to degrade carrier-bound hormone and by the schedule of their appearance in the haemolymph, the JH-esterases of type II can lower the JH level at the critical time for the induction of metamorphosis. Because non-hydrolysable JH analogues are considerably more active than JH itself in bioassays⁸, it is possible that the JH-esterases are the determining factor in the control of metamorphosis.

Table 2 Effect of *M sexta* carrier protein on cuticle deposition in cultured imaginal wing disks of *Plodia interpunctella*

[JH]* $\times 10^4\text{ M}$	Inhibition of cuticle deposition (%)		
	CP absent	$4 \times 10^{-9}\text{ M CP}$	$8 \times 10^{-9}\text{ M CP}$
0	0	0	0
1.7	15†	—	—
2.1	28†	50†	100†
4.2	30	70	—

In each experiment the mesothoracic wing disks were removed from 10 *Plodia* larvae weighing 12–15 mg. Each pair of disks was divided between experimental and control culture dishes. Fat body was removed from the same larvae and was present in all culture dishes. Either 200 or 400 μl of modified Grace's medium was used in glass micro-dishes containing 10^{-6} M β -ecdysone. Unless otherwise noted, 10 wing disks were used for each experiment. Imaginal disks in this culture system produced tanned cuticle which was scored using a dissecting microscope.

*The juvenile hormone was a mixture of geometric isomers of *Cecropia* juvenile hormone provided by Hoffman-LaRoche.

†Twenty disks were examined.

‡Forty disks were examined.

It is tempting to extend our conclusions to insects other than *M sexta* since a similar low molecular weight carrier protein has been detected in larvae of *P interpunctella*⁹. In addition, we have tentatively identified a low molecular weight carrier in *H cecropia* larvae. The timetable for metamorphosis in all holometabolous insects is very similar and thus a degradative enzyme specific for the hormone-carrier complex could indeed be universally involved in the control of metamorphosis.

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A neuroendocrine feedback mechanism in the insect moulting cycle

THE principles of neuroendocrine integration and regulation developed by the Scharrers^{1,2} are central to current concepts of the regulation of the hypothalamo-hypophyseal system and are applicable to all neurosecretory systems. The importance of these principles in the regulation of the analogous system in insects (the protocephalic corpus cardiacum-allatum system) has, however, been little studied. Here I illustrate certain general functional parallels in the regulation of the vertebrate and insect systems by reference to work on the regulation of the neurosecretory pathway controlling moulting in *Rhodnius prolixus*.

Classical studies^{3,4} have shown that moulting in fifth instar *Rhodnius* is initiated at feeding by the release of a neurosecretory 'brain hormone' which is necessary for 6–8 d after feeding if the moulting process is to proceed to ecdysis. After this 'critical period' the continued release of 'brain hormone' is unnecessary and the secretory behaviour of the medial neurosecretory cells (MNC), which produce it, changes at about this time⁵. The principles of neuroendocrine integration suggest that this change would be implemented after appropriate information had been received by the brain indicating the effectiveness of its released neurosecretory material (NSM). As the principal known effect of this NSM is stimulation of the production of the moulting hormone, ecdysone, experiments have been devised to determine whether the changes in the cyfological behaviour on the MNC about the time of the 'critical period' are mediated by the increased haemolymph titre of ecdysone. If this is the case, it should be possible to induce

them prematurely by providing the insect with a supply of ecdysone before its endogenous course of the hormone has become fully active

In fifth instar *Rhodnius* decapitated 8 d after feeding all known endocrine centres are removed except those producing ecdysone, and the release of ecdysone continues⁶ *Rhodnius* decapitated at 8 days therefore provided insects with intact cerebral neuroendocrine systems early in their moulting process (1 d after feeding) to a sustained prematurely high physiological titre of ecdysone, using parabiosis. When serial sections of the brain (stained with paraldehyde fuchsin) from insects after 7 d of parabiosis (that is, on the eighth day after feeding) were examined, the MNC had been induced to switch over from their normal sequence of cytological changes to those characteristic of the older insect⁷. In particular, a massive number of Type 2a cells is produced during a period when they are otherwise absent, and there is a corresponding decrease in the number of cell Types 2 and 1 (Table 1). Such changes are induced by the haemolymph of donor insects which have passed the 'critical period' but not by that of insects which have yet to reach it. Thus the changes observed in the MNC during the normal moulting cycle⁸ are not an immutable sequence of events but respond dramatically to changes in the composition of the circulating haemolymph.

More direct evidence that ecdysone is the factor responsible for these effects on the MNC has been sought using synthetic hormones. The brain was exposed to a prematurely elevated titre of synthetic ecdysone by injection of 1 µg β-ecdysone in 1 µl sterile Ringer solution at 1 d and 3 d after feeding. The two separate injections were given in an attempt to maintain high ecdysone titres from 1–8 d after feeding and minimise any inactivation of the hormone by the fat body⁸. The MNC were examined on the eighth day as before. Ringer-injected controls were normal but the MNC of insects receiving β-ecdysone were strikingly similar to those of insects exposed to the hormone using parabiosis (Table 1).

Ecdysone administration clearly influences the cytology of the MNC. The observed effects of ecdysone are not normally encountered before the 'critical period' but are characteristic of normal insects which have passed it⁸ and which contain the hormone^{9,10}. Therefore, the changes in the cytological behaviour of the MNC which occur at the 'critical period' in normal insects are probably brought about by ecdysone. This indicates a feedback relationship between the prothoracotrophic 'brain hormone' and ecdysone: the behaviour of the MNC producing the 'brain hormone' changes with the increased titre of ecdysone, the production of which it stimulates. These changes are normally accomplished at the 'critical period', after which the moulting process is independent of the brain but if a premature independence from the 'brain hormone' is experimentally impressed on the insect by an exogenous supply of ecdysone, the MNC behave as though the period during which such independence is normally achieved, had arrived.

Reasoning has been presented elsewhere that the cytological changes induced by ecdysone indicate that the hormone causes an inhibition of release of NSM from the MNC

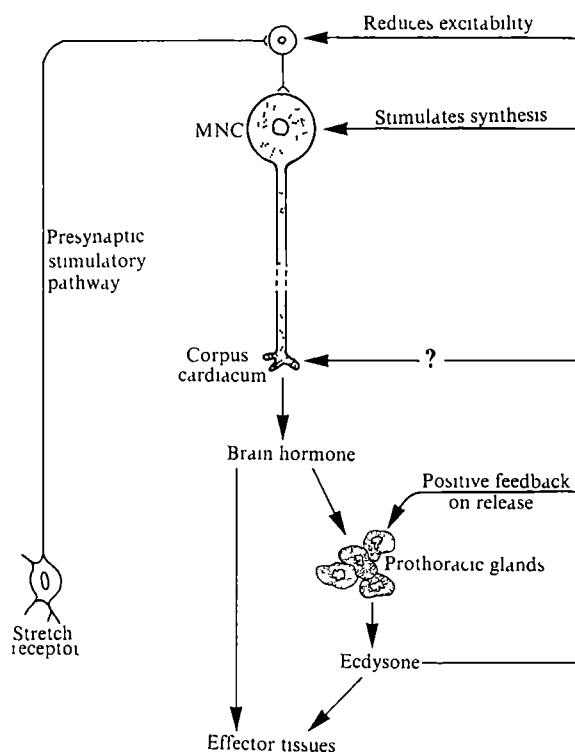


Fig 1 Schematic diagram of the proposed interactions between the cerebral neurosecretory system and ecdysone during moulting in *Rhodnius*

coupled with stimulation of synthesis of new material⁷. Hence the effect of ecdysone on the MNC is apparently a negative feedback on release of NSM coupled with a stimulation of synthesis. Such an arrangement possesses striking functional similarities with the so-called 'short-loop' feedback mechanisms familiar (though controversial) in the regulation of the anterior pituitary whereby adenohypophyseal hormones influence production of their respective releasing factors in hypothalamic nuclei^{10,11}. The adenohypophysis and prothoracic gland are comparable as endocrine effectors of neurohormones¹² and it now seems that both glands provide the neurosecretory cells which regulate them with humoral feedback information.

The following scheme suggests how this feedback mechanism may operate in the normal moulting cycle of *Rhodnius* (Fig 1). Abdominal distension following feeding excites stretch receptors which discharge to the brain^{4,13}, providing presynaptic stimulation of the MNC and causing release of NSM⁷. The principal effect of the NSM is to stimulate production of ecdysone which promotes moulting in the tissues. Ecdysone seems to have a positive feedback on its own production^{14,15}, thereby amplifying the hormonal instructions from the brain. By the time ecdysone production peaks, release of 'brain hormone' is greatly reduced⁵ and the head is no longer necessary for moulting⁴. This decline in 'brain hormone' release is not due merely to a decline in the afferent stimuli favouring release, however,

Table 1 Effects on the MNC of premature exposure to ecdysone by parabiosis and by injection of synthetic hormone

Cell type	Type 1	Type 2	Type 2a	Type 3	Type 4	Total
Normal insect at day 8	4.2 ± 0.2	6.4 ± 0.2	0.2 ± 0.1	3.2 ± 0.3	5.0 ± 0.0	19.1 ± 0.3
Parabiosis (from 1 to 8 d) to a decapitated insect producing ecdysone	1.8 ± 0.2	2.6 ± 0.3	5.8 ± 0.5	3.2 ± 0.4	5.3 ± 0.1	18.7 ± 0.4
Ecdysterone (1 µg) injected at days 1 and 3	2.1 ± 0.5	4.1 ± 0.6	6.4 ± 0.5	2.6 ± 0.3	5.0 ± 0.1	20.3 ± 0.6

Figures are the mean number (± s.e.) of the five cytologically distinct cell types present in the MNC on day 8 after feeding, following the treatments described

because the time course of the process is influenced by ecdysone, the increasing titre of which seems to act back on the brain where release is inhibited and synthesis of NSM promoted. Indeed, an engorged *Rhodnius* remains noticeably distended for several weeks after feeding and the abdominal stretch receptors show a maintained adapted discharge rate¹⁶. It follows that at the time when the effects of ecdysone on the MNC normally become apparent (at 6–8 d), neural stimuli favouring release would still be arriving at the brain and the neurosecretory system would have to 'decide' between these conflicting neural and humoral instructions. The significance of the MNC as the 'final common path'¹⁷ of this process of neuroendocrine integration is most obvious in the experiments where ecdysone is provided 1 d after feeding, the rate of release of NSM in response to the neural stimuli is normally very high at this time⁵ but these stimuli cease to be effective when ecdysone is provided. This suggests that the feedback effect of ecdysone may occur either directly on the MNC or on neurones concerned with their presynaptic control, to reduce their sensitivity to presynaptic stimulation (Fig. 1). Indeed, there is some evidence that ecdysone directly depresses the firing rate of insect central neurones¹⁸. Similarly, the effect of adenohypophyseal hormones on the hypothalamus may be on the cells elaborating releasing factors or on neurones controlling them^{10,11}. Steroid sensitivity is apparently not a general property of central neurones, however, as not all hypothalamic neurones show it¹⁹ and in *Rhodnius* some of the cell types in the MNC respond to ecdysone while others seem to be unaffected (Table 1).

The reported inhibition of release of NSM at the 'critical period' is thus explicable in terms of selective modulation of central excitability by ecdysone, the simultaneous promotion of synthesis by ecdysone must involve other mechanisms which are provisionally assumed to include direct action of ecdysone on the MNC (Fig. 1). Although tentative because of the indirect nature of the evidence, the above scheme illustrates the mutual interdependence of insect hormones and the importance of neuroendocrine integration in their regulation.

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Role of growth hormone in glycosaminoglycan synthesis by articular cartilage

LITTLE is known of the factors which regulate the turnover of the glycosaminoglycans, chondroitin sulphate and keratan sulphate,

in articular cartilage. Studies using costal cartilage¹ suggest that growth hormone (somatotrophin) has little direct action on glycosaminoglycan synthesis. Instead its action is thought to be mediated by the liver² which produces a different hormone, somatomedin, previously known as 'sulphation factor' because it stimulates the incorporation of ³⁵SO₄ into costal cartilage.

These conclusions are not necessarily true for articular cartilage. Denko and Bergenstal³ found differences between the incorporation of ³⁵SO₄ by costal cartilage and the cartilage of the tibial 'cap' in young rats. For example, following hypophysectomy, incorporation of ³⁵SO₄ by costal cartilage was depressed, but no change was observed for the tibial 'cap'.

That such differences should exist is not surprising, for although costal and articular cartilage resemble each other in structure, their function is entirely different. Furthermore, it is necessary to distinguish between articular cartilage and epiphyseal cartilage and the tibial 'cap' is unsatisfactory because it includes both.

We carried out two separate experiments to investigate the action of growth hormone on articular cartilage. *In vitro* studies enabled us to investigate the action of bovine growth hormone on bovine articular cartilage. Of necessity we had to use smaller animals for *in vivo* studies and we selected the rat, as bovine growth hormone is known to be biologically active for this species.

In the *in vivo* studies bovine growth hormone (NIH-GH B17) was given by subcutaneous injection for 14 d to mature male rats. During this treatment these 'plateaued' rats showed a gain in weight, compared with a control group which showed a slight fall. After 14 d, each animal was given 100 μ Ci ³⁵S-labelled Na₂³⁵SO₄ by intraperitoneal injection and killed 24 h later. The distal femora and proximal tibiae from each animal were decalcified and sections through their joint surfaces examined by the method of autoradiography. There was no detectable difference between the group treated with growth hormone and the controls with regard to labelling of the articular cartilage. Slivers of articular cartilage were cut from the proximal humerus of the same two groups of animals for quantitative study. These samples were weighed by an electrobalance and dissolved by boiling with 23 M formic acid, radioactivity was determined using a Packard Tri-Carb liquid scintillation counter. Counts for each sample were divided by their weight to give specific activity. This showed no significant difference between the two groups.

In such an *in vivo* study, it is not possible to distinguish between the effects of growth hormone itself and somatomedin which is presumably released by the liver in response to growth hormone injections. These factors were investigated separately *in vitro*, using a medium containing somatomedin prepared by previous incubation of growth hormone with liver slices⁴. Optimal conditions for glycosaminoglycan synthesis were obtained by organ culture of bovine articular cartilage in 20% oxygen and using a chemically defined medium⁵. The articular cartilage was obtained from 6-month-old calves, being from the same species as the growth hormone (NIH-GH B17).

Organ culture of articular cartilage was performed simultaneously using eight culture chambers, divided into four groups, differing only in the nature of the culture medium⁴. Group A, Eagle's MEM, group B, Eagle's MEM containing bovine growth hormone (concentration 1.0 μ g ml⁻¹), group C, Eagle's MEM incubated beforehand with liver slices, group D, Eagle's MEM plus bovine growth hormone (concentration 1.0 μ g ml⁻¹) incubated beforehand with liver slices.

Four samples of cartilage, each about 1 mg dry weight, were removed from each chamber after 24, 48 and 72 h. This amount of material is adequate for fractionation of glycosaminoglycans on the microscale⁶.

Two separate experiments were performed, using different donor animals to provide the articular cartilage. Samples were pooled from each of the groups, A, B, C and D, so that the final values represent analysis of 16 separate pieces of articular cartilage for each group.

Table 1 Specific activities of fractions from CPC cellulose microcolumns

Eluting solution	24 h				48 h				72 h			
	A	B	C	D	A	B	C	D	A	B	C	D
0.3 M NaCl	1,256	2,077	959	748	1,314	636	407	828	1,374	1,297	664	581
0.25 M MgCl ₂	4,666	4,197	1,289	1,568	4,111	1,381	788	756	2,668	2,089	930	1,477
0.3 M MgCl ₂	1,898	1,098	1,008	1,201	1,918	492	493	523	1,550	563	598	877
0.35 M MgCl ₂	979	667	519	542	872	313	189	321	788	334	469	371
0.4 M MgCl ₂	882	479	415	505	588	134	150	201	454	276	237	260
0.45 M MgCl ₂	869	495	309	474	433	111	114	115	492	198	208	189
0.5 M MgCl ₂	1,377	1,075	472	472	427	120	134	100	506	196	325	263
0.6 M MgCl ₂	4,305	2,213	1,794	2,215	778	165	262	100	499	228	574	676
2.0 M MgCl ₂	1,585	2,102	2,430	2,144	364	125	556	983	259	075	510	948
6 M HCl	375	238	342	263	106	283	126	046	228	0	279	318
Total chondroitin sulphate	1,290	828	533	630	689	245	185	131	716	315	337	332
Highly sulphated keratan sulphate	954	828	1,233	895	273	170	354	522	240	330	388	672

The values shown are means of two calculations of specific activity (c.p.m. divided by hexosamine content in μg) for each fraction eluted from the CPC cellulose microcolumns. The different experimental groups, A, B, C and D are explained in the text. Chondroitin sulphate of increasing chain length is eluted by the 0.25 M to 0.6 M MgCl₂ solutions and highly sulphated keratan sulphate by the 2.0 M MgCl₂ and 6.0 M HCl fractions. The mean specific activity for these two glycosaminoglycans was determined by dividing the sum of counts for each of their fractions by the sum of the hexosamine content of these fractions.

After their removal from the culture chambers, the cartilage samples were incubated in Tyrode's solution, containing $0.5 \mu\text{Ci } ^{35}\text{SO}_4 \text{ ml}^{-1}$. After 1 h they were removed and further enzyme activity prevented by immersion in monoiodoacetic acid. The samples were washed to remove unbound $^{35}\text{SO}_4$, dehydrated in acetone and dried in air to constant weight. Following digestion by papain, the glycosaminoglycans were fractionated on cetyl pyridinium chloride (CPC) cellulose microcolumns⁶, using eluting solutions as shown in Table 1. All analyses were performed in duplicate. Chondroitin sulphate is eluted by the magnesium chloride solutions 0.25 M to 0.6 M and highly sulphated keratan sulphate by 2 M magnesium chloride and 6 M HCl. The first fraction obtained by elution with CPC contains keratan sulphate with glycopeptides and some unbound sulphate. These components were separated using ecteola cellulose microcolumns⁷, keratan sulphate being eluted by 0.4–1.2 M NaCl solutions. Characterisation of these fractions from calf articular cartilage has been previously reported using electrophoresis on cellulose acetate membranes⁸. Each fraction

was divided into aliquots for determination of hexosamine content and scintillation counting.

The results shown in Tables 1 and 2 are indices of the synthesis rate of each fraction, that is, c.p.m. divided by the weight of hexosamine in micrograms ('specific activity').

The addition of growth hormone to the culture medium (group B) resulted in diminished incorporation of $^{35}\text{SO}_4$ for all fractions after each of the time intervals studied. This applies equally to all fractions of chondroitin sulphate (0.25 M to 0.6 M MgCl₂ fractions) and keratan sulphate (0.4 M to 1.2 M NaCl fractions plus the 6 M HCl fractions). A similar depression of $^{35}\text{SO}_4$ is seen for group D (previous incubation of medium with growth hormone plus liver slices), but with the exception of highly sulphated keratan sulphate (2.0 M MgCl₂ and 6 M HCl fractions from the CPC columns). After 48 and 72 h, this highly sulphated keratan sulphate showed an enhanced incorporation of $^{35}\text{SO}_4$, presumably in response to somatomedin, as no such change was seen for group B or group C (previous incubation with liver slices only).

The design of this experiment was very similar to that of

Table 2 Specific activities of fractions from ecteola cellulose microcolumns

Eluting solution	24 h				48 h				72 h			
	A	B	C	D	A	B	C	D	A	B	C	D
H ₂ O	0	0	0	0	0	0	0	0	171	900	0	173
0.02 M HCl	0	0	0	0	066	0	0	0	1,928	354	0	040
0.3 M NaCl	829	477	303	224	1,106	398	086	188	2,538	1,589	347	484
0.4 M NaCl	1,339	813	422	236	2,193	586	353	375	2,519	1,047	384	413
0.5 M NaCl	729	717	271	057	1,450	329	176	240	1,251	466	228	294
0.6 M NaCl	362	217	0	0	142	167	074	099	443	148	053	115
0.7 M NaCl	141	030	0	0	179	0	0	0	204	0	072	034
0.8 M NaCl	117	211	0	0	121	0	0	0	060	0	0	074
1.2 M NaCl	0	0	0	0	0	0	0	0	0	0	0	0
6 M HCl	0	0	0	0	0	0	0	0	0	0	0	0
Total keratan sulphate	493	298	124	062	708	157	092	109	1,210	402	169	197

The values shown are means of two calculations of specific activity for each fraction eluted from the ecteola cellulose microcolumns on refractionation of the CPC fractions obtained from the CPC cellulose microcolumns. Keratan sulphate is eluted by the 0.4 M to 1.2 M NaCl fractions and a mean specific activity was determined by dividing the sum of the counts for these fractions by the sum of their hexosamine content. Unbound $^{35}\text{SO}_4$ was removed from the ecteola cellulose columns by previous incubation with 0.1 M NaCl, repeated six times to give six fractions which had no significant hexosamine content. The zero for some of the fractions shown in this table represents a count below that of a standard blank salt solutions of the same molarity.

McConaghey⁴, who found that incorporation of ³⁵SO₄ by costal cartilage was slightly increased by the same purified growth hormone and markedly increased by somatomedin, prepared using liver slices. It is clear that articular cartilage behaves quite differently in its response to growth hormone, which, *in vitro*, depresses glycosaminoglycan synthesis. The response of articular cartilage to somatomedin was similar to that of costal cartilage only for highly sulphated keratan sulphate. It is very likely, however, that the medium used in group D still contains growth hormone not metabolised by the liver slices. This residual growth hormone could have obscured the action of somatomedin on the synthesis of chondroitin sulphate and keratan sulphate.

Whatever the effect of somatomedin, there can be no doubt that growth hormone itself has a direct action on articular cartilage *in vitro*. That action is to decrease glycosaminoglycan synthesis.

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Purification of human uterine progesterone receptor

It is now generally accepted that before the physiological response to the androgens¹, oestrogens², glucocorticoids³ and progesterone⁴⁻⁷ can be manifested, these hormones must complex with specific receptors in their target tissue. Since progesterone is important both in the mechanisms associated with transport of the ovum through the oviduct^{8,9} and in preparing the endometrium for implantation of the blastocyst¹⁰, a greater understanding of the action of the hormone-receptor complex at the molecular level will lead to a more detailed knowledge of the mechanisms involved in reproductive biology. This understanding may be utilised to develop new contraceptive methods and to investigate the genesis and treatment of endometrial adenocarcinoma.

To study the molecular events associated with the function of progesterone and to determine the topography of the receptor site, it is necessary to obtain the receptor-hormone complex in a pure form. We report here the isolation and purification of the progesterone receptor from human uterus. A similar receptor has also been studied from the human oviduct (C. A. I., unpublished), and translocation and binding to oviduct nuclei has been demonstrated. Purification was achieved using a combination of ammonium sulphate fractionation, affinity chromatography and ion-exchange chromatography. The overall purification from the 105,000g supernatant fraction from a

crude tissue homogenate is approximately 8,000-fold. Since the cytosol fraction is an approximately fivefold purification over the whole cell we estimate a 40,000-fold purification for the progesterone receptor with respect to the intact cell. The concentration of progesterone binding sites in the crude cytosol is 3×10^{-8} M per mg protein. The pure receptor sediments at 3.7S on sucrose gradient centrifugation and migrates as a single band of molecular weight 110,000 on SDS polyacrylamide gel electrophoresis. It has not yet been determined, however, whether more than one subunit of the same molecular weight is present. These data will be reported in detail elsewhere.

The specificity of the ammonium sulphate-purified receptor (Table 1) showed the following relative affinities: progesterone > 11-desoxycorticosterone > 5 α -dihydroprogesterone > 5 β -

Table 1 Comparisons of hormone binding to the progesterone receptors from human, chick and hamster target tissues

	Human	Chick ⁸	Hamster ⁹
Progesterone	100	100	100
5 α -Dihydroprogesterone	12	86	20
5 β -Dihydroprogesterone	6	15	4
11-Desoxycorticosterone	28	94	64
Substance S	7	7	11
Corticosterone	5	3	3
Cortisol	<1	<1	<1
Testosterone	2	5	4
Oestradiol	<1	<1	1

Tissues were segmented and rinsed in ice-cold saline solution, weighed and homogenised in four volumes (w/v) TESH buffer, 0.01 M Tris-HCl, 1.0 mM Na₂EDTA and 0.012 M thioglycerol (pH 7.4). The homogenate was centrifuged at 27,000g for 10 min and the supernatant was re-centrifuged at 105,000g for 1 h to obtain the cytosol fraction. Glycerol was added to the buffer to a concentration of 40% when human uterine tissue was used. For the competition assay a series of tubes were prepared containing cytosol, ³H-progesterone and unlabelled steroid competitors in TESH buffer. The assay tubes were incubated at 0°C for 16-20 h and ³H-progesterone binding was measured by charcoal adsorption. Each assay point was determined twice. A standard curve for the competition of unlabelled progesterone was included in each assay using five progesterone concentrations usually in the range 3.5×10^{-9} to 7.1×10^{-7} M. Four or five concentrations of each competitor were tested using a range between 3×10^{-9} and 7×10^{-7} M. The competitor concentrations were chosen to provide a linear portion on a semi-log plot which would cross the point of 50% competition. From this plot, the concentrations of unlabelled progesterone and of steroid competitors which reduced ³H-progesterone binding by 50% were determined. These quantities were converted to molar concentrations and the effectiveness of the competition was established using the ratio: unlabelled progesterone concentration for 50% competition/competitor concentration for 50% competition. This ratio was multiplied by 100.

dihydroprogesterone > corticosterone > testosterone >> oestradiol > cortisol. The relative affinity of cortisol shows that the most probable contaminant, cortisol binding globulin, is absent. A comparison of these specificities with those obtained from crude cytosol receptors of chick oviduct¹¹ and hamster uterus¹² (Table 1) shows that the partially purified human uterine receptor is the more selective. It was not possible to determine specificity by competitive assay for progesterone binding sites at subsequent steps in the purification since the receptor is saturated with labelled progesterone during its elution from the affinity column. Because of the lability of the receptor, it has not yet been possible to remove the hormone and retain the native activity of the receptor. Scatchard analysis shows, however, that only molecules containing binding sites specific for progesterone are retained by the affinity resin.

The affinity resin was prepared by coupling denatured bovine serum albumin to cyanogen bromide-activated Sepharose¹³ followed by reaction with 11-desoxycorticosterone hemisuccinate in the presence of 1-ethyl-3-(3-dimethylaminopropyl)

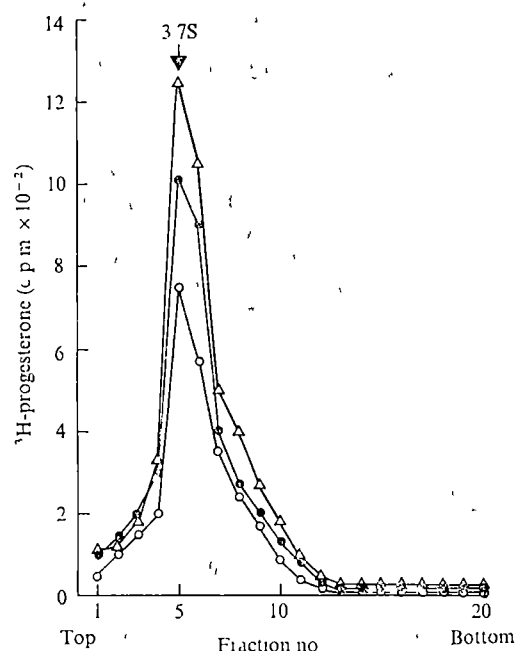


Fig. 1 Sucrose gradient ultracentrifugation analyses of ^3H -progesterone-treated Δ , human uterine cytosol; \bullet , receptor obtained after affinity chromatography; \circ , nuclear extract (0.4 M KCl) from human oviduct. Samples (0.2 ml) were layered on linear 5–20% (w/v) sucrose gradients made up in 10% glycerol, containing 0.05 M Tris, 1 mM EDTA and 0.01 M thioglycerol at pH 7.5, and then centrifuged for 17 h at 216,000g. ^{14}C -ovalbumin (3.7S) was used as a sedimentation standard.

carbodiimide hydrochloride. After thoroughly washing each millilitre of resin with 200 ml dioxane followed by 21.80% methanol/water, the capacity of the resin is 0.21 nmol per ml packed resin and its dissociation constant for the receptor is 10^{-9} M (the dissociation constant of progesterone for its receptor is $\sim 10^{-9}$ M). Cortisol (10^{-6} M) was added to the ammonium sulphate-precipitated receptor prior to incubation with the affinity resin, so that binding of the resin to corticoid receptors and possibly certain steroid metabolising enzymes would effectively be negated. After incubation for 12 h at 2°C each millilitre of resin was washed with 200 ml of buffer containing a high salt concentration (0.4 M KCl) to remove relatively loosely associated proteins. Elution of the receptor in batches was accomplished by incubating at 20°C for 30 min in the presence of excess labelled progesterone. The macromolecular bound hormone was isolated by gel filtration and applied to a DEAE-cellulose column and eluted with a salt gradient. The receptor-progesterone complex was eluted in 0.2 M KCl, dialysed, lyophilised and applied to an SDS polyacrylamide gel. Electrophoresis revealed a single band of molecular weight 110,000.

The dissociation constants for progesterone ($K_D \sim 10^{-9}$ M) and the relative affinities of various steroids for the crude and the partially purified receptor are almost identical (Table 1). The crude, the partially purified and the pure receptor all show

similar physical properties: the macromolecule sediments at 3.7S (Fig. 1) and is eluted from phosphocellulose by 0.17 M KCl, from DEAE-cellulose by 0.2 M KCl. A nuclear extract obtained after preincubation of nuclei with labelled receptor also sediments at 3.7S (Fig. 1). Collectively, the data therefore confirm the integrity of the pure progesterone receptor.

Incubation of human oviduct nuclei with receptor- ^3H -progesterone complex, both at 0°C and 37°C, showed translocation was occurring. Extraction of the nuclei with a high salt buffer (0.4 M KCl) followed by sucrose gradient centrifugation of the extract demonstrated macromolecular bound hormone sedimenting at 3.7S. It is not surprising that nuclear binding occurs at both temperatures since it has been shown previously in the chick oviduct system that activation of the progesterone-receptor complex by ammonium sulphate alone is sufficient to effect translocation and binding at 0°C (ref. 14). Human oviduct rather than endometrial nuclei were used because of the greater amount of tissue available combined with ease in obtaining pure nuclei.

For the first time, therefore, we have demonstrated the purification of a human uterine progesterone receptor (Table 2), together with its translocation and binding to human target nuclei. Characterisation of the receptor site may enable us to design specific agents to block progesterone binding at this site thus precluding the physiological action of this hormone. In addition, we now have the opportunity to study in detail the mechanism of action of progesterone.

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Table 2 Purification of human uterine progesterone receptor

	c.p.m./mg protein	Yield (%)	Purification (-fold)
Cytosol	2×10^4	—	—
Ammonium sulphate (30%)	8.5×10^4	30	4.3
Affinity column eluent	5.4×10^7	100	2,700
DEAE cellulose-0.2 M KCl	1.65×10^8	10	8,300

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Is potassium conductance of cardiac Purkinje fibres controlled by $[Ca^{2+}]_i$?

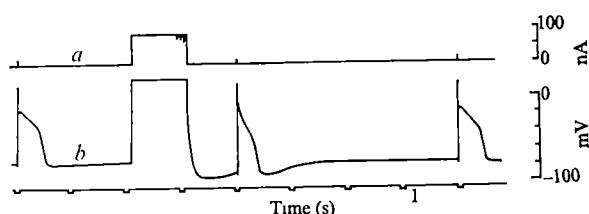
ELEVATED extracellular Ca^{2+} concentration¹, prolonged treatment with high doses of cardiac glycosides², and metabolic inhibition³ shorten the action potential of cardiac muscle. In these conditions the intracellular free Ca^{2+} may be increased and an increase in intracellular Ca^{2+} could produce shortening of the action potential either by affecting the Ca^{2+} inward current or by increasing the potassium permeability⁴. The latter mechanism has been observed in red blood cells⁵ and in the neurones of some species⁶⁻⁸. Here I give the results of studying the effect of iontophoretically injected Ca^{2+} in cardiac Purkinje cells. Such injection led to membrane hyperpolarisation and considerable shortening of the action potential, suggesting that the level of intracellular free Ca^{2+} can affect the potassium permeability in cardiac muscle.

The diffusion coefficient of radiocalcium in skeletal muscle fibres seems to be 100 times less than that in water⁹. This suggests that an effect produced by the electrophoretic injection of Ca^{2+} can only be observed within a small region round the injection site. To ensure that the tip of the microelectrode recording resting and action potentials was placed in the region of elevated $[Ca^{2+}]_i$, in most experiments a single electrode filled with 1 M $CaCl_2$ was used both for injection and for recording¹⁰. A second intracellular electrode (filled with potassium citrate) served to stimulate the preparations at a rate of 15 min⁻¹.

In Fig 1*b*, the first action potential (AP) illustrates the control values (resting potential (V_m), -76 mV, plateau potential, -16 mV, duration of the action potential (APD), 635 ms). Thereafter an inward directed current pulse (Fig 1*a*) injected about 0.1 μ Ci from the Ca^{2+} filled electrode, this current depolarised the preparation to +20 mV for 1 s. When the injecting current was switched off the membrane re- and hyperpolarised (V_m -95 mV). One second after injection, the second AP was elicited. The plateau began at a potential more positive (-7 mV) and was shorter than that of the control, the APD being reduced to 380 ms. From the plateau, the membrane repolarised (with almost unchanged speed) to a high negative (-91 mV) diastolic potential. Within the next 2 s V_m declined like an enhanced pacemaker potential and 5 s after injection both V_m and the AP were almost identical with the control.

It could be argued that injecting the current produces not only an increased $[Ca^{2+}]_i$, but also a strong activation of the potassium conductance g_K due to the large depolarisation⁷. To exclude the latter possibility, in five experiments the Ca^{2+} injecting electrode was replaced by a double-barrelled electrode, one side filled with $CaCl_2$, the other with 3 M KCl. The current was injected between the $CaCl_2$ barrel and second intracellular microelectrode filled with potassium citrate without an earth

Fig 1 Effect of intracellular iontophoretic injection of +0.1 μ Ci (a) from a microelectrode filled with 1 M $CaCl_2$ solution on transmembrane resting and action potential (b). Experiment on sheep Purkinje fibre, superfused by oxygenated Tyrode solution containing (mM): 150 NaCl, 4 KCl, 0.9 $CaCl_2$, 1 $MgCl_2$, 5 glucose and 10 Tris-maleate buffer, pH 7.4, temperature 37°C. Chart recording, the action potential overshoots are attenuated. The depolarisation during injection was measured by the non injecting K-citrate electrode.



(battery-driven current pump). A third intracellular microelectrode was used for stimulating the preparation. A current of +100 nA out of the $CaCl_2$ capillary, hyperpolarised the membrane from -85 to -92 mV and 0.8 s after the end of injection the APD was shortened from 540 ms (control) to 320 ms. If, after injecting the Ca^{2+} , the membrane was clamped to the control level (-85 mV) for 500 ms, nearly the same reduction of APD (from 540 to 310 ms) was observed.

The resistance of the electrode increased during injection. When more than 0.5 μ Ci were applied the current frequently decreased (R_{ei} more than 800 M Ω , saturation of the current pump⁷). It was then difficult to separate the Ca^{2+} induced hyperpolarisation from an increase in tip potential. The stability of the electrodes was better when they were filled with a Ca^{2+} -EGTA buffer solution (electrodes filled with 0.09

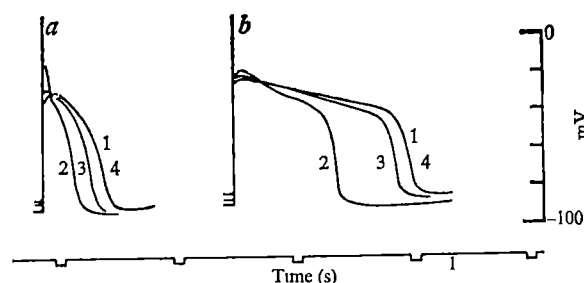


Fig. 2 Transmembrane action potentials. Each panel shows photographically superimposed chart recordings of control action potentials (trace 1 in *a*, *b*) and after iontophoresis *a*, Injection of +0.3 μ Ci from a microelectrode filled by Ca^{2+} -EGTA buffer solution Tyrode with $K_o = 5.4$ mM. Action potential 0.4 s (trace 2), 0.8 s (trace 3) and 2.5 s (trace 4) after the end of the injecting current pulse. *b*, Injection of +0.3 μ Ci as in *a*, but at a lower K^+ concentration (4 mM) in the Tyrode solution. Action potential 0.8 s (trace 2), 1.5 s (trace 3) and 3 s (trace 4) after the end of the injecting current pulse.

M $Ca(OH)_2$, 0.1 M EGTA, and 0.1 M Tris, resistance of the electrodes 120-150 M Ω)⁸.

Figure 2*a*, trace 2, shows an AP 0.4 s after injection of +0.3 μ Ci from an electrode filled with Ca^{2+} -EGTA buffer. The injection caused hyperpolarisation and a reduction of the APD from 550 ms (trace 1, control) to 250 ms (trace 2). The configuration of the AP was altered similar to that in Fig. 1. For constant charges of the injected Ca^{2+} , the shortening of the APD was more pronounced the shorter the delay between injection and AP. Thus, 0.8 s after injection, the APD was shortened only by a small amount (Fig 2*a*, trace 3) and 2.5 s after injection the AP was identical to the control (Fig. 2*a*, traces 1 and 4). This normalisation of the APD could be fitted by a single exponential. From 22 experiments, a time constant of 0.5 ± 0.2 s was calculated for the decay.

In Fig 2*b* the Tyrode solution contained a K^+ concentration somewhat smaller (4 mM) than in Fig 2*a* (5.4 mM). Injection of +0.3 μ Ci produced hyperpolarisation of V_m and shortening of APD as in Fig. 2*a*. Both effects however, were more pronounced and lasted longer (time constant for the decay, 1.0 ± 0.2 s, $n, 15$).

When the time interval between injection and AP was constant, the APD was shorter the larger the amount of the injected Ca^{2+} . Currents less than 10 nA did not affect the APD, independent of their duration (presumably the cell can rapidly extrude or sequester such an amount). The formulation of a quantitative relationship was not, however, possible with the present data.

The shortening of the APD on the injection of Ca^{2+} could be explained by a reduction of the Ca^{2+} driving force diminishing

the slow inward current? Viewed in conjunction with the strong hyperpolarisation, however, the most likely explanation is that elevated $[Ca^{2+}]$, increased the potassium conductance g_K .

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Role of foetal adrenocorticotrophin during parturition in sheep

It is now well accepted that the lamb foetus plays an important role in the mechanisms which initiate its own delivery. This conclusion, is based on several experimental observations. Foetal hypophysectomy or adrenalectomy delays parturition^{1,2}, infusions of adrenocorticotrophin (ACTH) or cortisol, which is the major glucocorticoid in this species, will precipitate premature delivery when infused into the foetus³, normal delivery is preceded by an increase in the concentration of corticosteroids in the foetal blood^{4,5} and an increased production of cortisol by the foetal adrenal⁶.

The simplest hypothesis to explain these changes is that an increase in foetal pituitary secretion of ACTH stimulates the production of cortisol from the foetal adrenal. The cortisol is thought to act on placental enzyme systems to bring about several changes, in particular an increase in maternal plasma oestrogen concentrations^{7,8}, and maternal oestrogens have been shown to rise sharply in the last few hours before delivery⁹. This increase also occurs after the administration of ACTH or glucocorticoids to the foetus⁷.

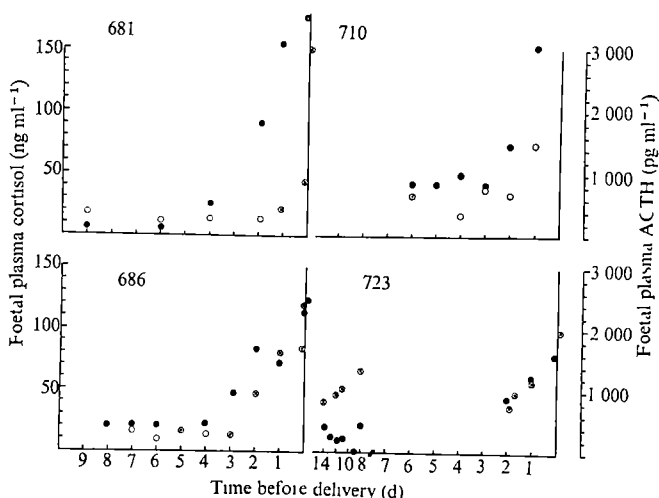
The results obtained from the experiments involving foetal hypophysectomy, however, do not prove that an increased secretion of ACTH by the foetal pituitary is necessarily involved in the initiation of parturition. It is possible that foetal hypophysectomy may delay parturition by lowering foetal plasma ACTH concentrations and thus impairing adrenal maturation and the development of adrenal responsiveness to other stimuli of either foetal or maternal origin. These alternative explanations have been discussed in detail elsewhere⁶. The changes in foetal plasma corticosteroid and ACTH concentrations in the period before parturition have been studied in the foetal sheep using indwelling vascular catheters¹⁰. Foetal plasma ACTH concentrations were reported to be very labile, 100-1,800 pg ml⁻¹, and the authors concluded that when average values were considered there seemed to be a rise in ACTH during the last days before delivery. Sequential values from the same animal for more than three days were available in only two foetal sheep which were delivered vaginally and one of the two ewes was subjected to three bouts of experimental hypoxia. It therefore seemed important to obtain further data* from undisturbed foetuses during late gestation and parturition. The experiments reported here were carried out to relate changes in plasma ACTH and cortisol concentrations both to each other and to the timing of delivery.

Seven ewes with singleton pregnancies of 124-130 d gesta-

tional age were used in the present investigation. A foetal venous catheter was introduced into each leg through the lateral tarsal vein. Foetal blood samples were taken at least once daily, if obtainable, throughout the experimental period for the assessment of foetal condition as indicated by blood gas and pH analysis, but no samples for hormone estimations were taken for at least 48 h after surgery. In all samples analysed for ACTH and cortisol concentration, foetal pH was within the normal range (7.32-7.41) except for samples taken during the final stage of labour when the usual fall in foetal pH was observed. Blood gas tensions were normal. Foetal blood was taken into cold syringes, centrifuged immediately and the plasma stored at -20°C. All estimations were performed within 1 month of sampling. Total plasma corticosteroids were measured in most samples by competitive protein binding without chromatographic separation¹¹. Some samples taken from foetuses at different days before delivery were separated on a Sephadex LH20 system¹². When this was done, cortisol accounted for more than 80% of the total corticosteroids as measured by competitive protein binding irrespective of gestational age. This finding is in agreement with the pattern of steroid secretion found when foetal adrenals of different ages are incubated *in vitro*¹³. Corticosteroid concentrations have therefore been designated as cortisol in the present study. Plasma ACTH was measured by radioimmunoassay after extraction on to porous glass¹⁴. Plasma samples of 0.5-1.0 ml were extracted and Third International Standard ACTH (31WS)¹⁵ used as both standard (unlabelled) and iodinated (labelled) hormone.

Two foetuses were each sampled four times over 48 h on days 130-132 of gestation to obtain data on foetal plasma ACTH concentrations before they were used for a separate experiment. Four foetuses were sampled up to the spontaneous delivery of a live lamb (Fig 1). One further foetus was sampled every 6 h (0000, 0600, 1200, and 1800) for 4 d. A

Fig 1 Plasma cortisol (●) and ACTH (○) concentrations over the last 14 d of intrauterine life in four chronically catheterised foetal sheep. Although the foetal catheters were patent, it was impossible to obtain blood samples from S723 between 8 and 2 d before delivery. S681 delivered at 147 d gestational age, 11 d after catheterisation, S686 delivered at 133 d gestational age, 9 d after catheterisation, S710 delivered at 139 d gestational age, 11 d after catheterisation, S723 delivered at 151 d gestational age, 23 d after catheterisation.



total of 40 ml of blood was removed in this period and therefore blood loss may have been a contributory factor to the subsequent death of this foetus *in utero*. It was equally possible that death occurred *intra partum* as the increase in foetal plasma cortisol was followed by a rise in maternal plasma oestrogen concentration while the foetus was still alive.

In none of the five foetuses which were sampled sequentially was there any significant increase in foetal plasma ACTH before the preparturient rise in foetal plasma cortisol concentration. In three of the animals, plasma ACTH concentrations were low in the period up to 96 h before delivery (242.5 ± 18.4 pg ml⁻¹, mean \pm s.e.m., $n = 16$). Foetuses 686 and 681 were in this group. Mean plasma ACTH concentration in the other foetuses was 691.8 ± 127.3 pg ml⁻¹ ($n = 9$) in this same period (foetuses 723, 710). For all five foetuses the mean plasma concentration of ACTH for the period up to 4 d prepartum was 435.8 ± 67.6 pg ml⁻¹ ($n = 25$). In the two further foetuses sampled four times between 130 and 132 d, mean plasma ACTH concentrations were 177 and 203 pg ml⁻¹.

In contrast to the absence of an increase in foetal plasma ACTH before the prepartum rise in foetal plasma cortisol concentration, there was a pronounced increase in foetal plasma ACTH concentration in the last 24 h of foetal life. In one of the two foetuses which initially had a low plasma ACTH concentration (681) ACTH rose to 882 pg ml⁻¹ in the last 6 h of intrauterine life. In the other foetus (686), ACTH rose to 1,680 pg ml⁻¹ 40 min before delivery. There was a further rise to 2,380 pg ml⁻¹ 5 min before delivery. Although strong, coordinated uterine contractions only occur in the last 6 h or so of gestation in the ewe, contractions are gradually increasing in frequency and amplitude over the last 24 h of foetal life. It may be that stimulation of the foetus by uterine contraction is one of the causes of the increase in plasma ACTH that occurs at this time. The ACTH released during this period is probably the cause of the rapid rise in cortisol concentration which occurs in the last few hours of foetal life. Immediately after delivery plasma ACTH reached 3,001 and 1,948 pg ml⁻¹ in two lambs (681 and 723).

We would emphasise that results which contain only single daily observations for foetal plasma ACTH must be viewed with caution for two reasons. First, it is well known that in the adult ACTH is secreted in an episodic manner¹⁶. The results obtained from the foetus which was sampled every 6 h showed that there were changes in both foetal plasma cortisol and ACTH concentrations during the day and there was some indication of a nyctohemeral rhythm in circulating concentrations. It may be that the total daily secretion of ACTH by the foetus increases before foetal cortisol rises. Such a mechanism would explain the increased sensitivity of the foetal adrenal which occurs just before parturition¹³, as well as the increased secretion of foetal corticosteroids^{6,13}. To investigate this possibility it will be necessary to sample the foetus more frequently. To avoid haemorrhagic stress an increase in the frequency of sampling will require the availability of the cytochemical assay for ACTH¹⁷. Second, in the adult mammal it is known that cortisol will exert a negative feedback on ACTH secretion at both physiological and pharmacological concentrations¹⁸. Thus, the high plasma corticosteroid concentrations immediately before delivery may be exerting a negative feedback action on ACTH secretion. The final level of ACTH secretion will depend on the summation of any such negative feedback and the positive drives on the hypothalamo-hypophyseal-adrenal axis.

In conclusion, simultaneous measurements of plasma ACTH and cortisol concentrations have been made on sequential blood samples obtained from seven chronically catheterised foetal sheep. Five of these foetuses were sampled sequentially for several days before delivery. The basal ACTH concentrations varied between animals. This variation in basal concentration cannot at present be explained. On the basis of the present data there was no evidence for an increase in foetal plasma ACTH before the preparturient rise in foetal plasma cortisol.

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Novel 4-hydroxycoumarin anticoagulants active against resistant rats

FIELD populations of common rats (*Rattus norvegicus*) resistant to anticoagulant rodenticides (for example, Warfarin) have appeared¹ in Scotland, Denmark, England and Wales, and more recently on the American continent. This resistance seems to be associated with a reduction in the efficiency of vitamin K metabolism² and is an inheritable factor controlled by a single dominant autosomal gene. Various theories have^{1,2} been advanced to explain the underlying mechanism. Resistance to other anticoagulants has now appeared, that to coumatetralyl being the least effective¹.

Reduction of the carbonyl group of Warfarin and replacement of the terminal methyl group with various substituted phenyl groups produces compounds showing increased anticoagulant activity^{4,5}. We have synthesised compounds of this type (compounds 1 and 2, Table 1) and have found that activity was only demonstrated in normal rats and not in the resistant strain.

Comparison of the structures of Warfarin and coumatetralyl suggested that the activity of the latter in resistant rats might be due to the manner in which, in the tetralin moiety, the four carbon atoms of the saturated ring are held adjacent to the unsaturated ring, whereas the four carbon atoms in the side chain of Warfarin can assume many conformations other than those adjacent to the benzene ring. Compounds were therefore synthesised containing substituted phenyl groups attached by a three carbon chain to a 4-hydroxy coumarin moiety, the three carbon chain being part of a saturated ring fused to a benzene ring. Examples of such are the 3-(3-*p*-substituted-1,2,3,4-tetrahydronaphth-1-yl) 4-hydroxycoumarins and related compounds shown in Table 1.

Several conclusions may be drawn from these results

Table 1 The activity of 3-(3-*p*-substituted phenyl-1,2,3,4-tetrahydronaphth-1-yl)-4-hydroxycoumarins and related compounds in normal (Wistar) and homozygous resistant Welsh strain *Rattus norvegicus*

No	Structure	Prothrombin ED ₅₀ mg kg ⁻¹		Resistance factor	Bait trial	
		Wistar	Resistant		Concentration (p.p.m.)	Mortality (%)
1		0.4	> 20	> 50	—	—
2		1.2	> 25	> 20	—	—
		1.2	25	20	—	—
	X R' R ²					
3	CH ₂ H 4-methyl	> 2.0	—	—	—	—
4	CH ₂ H 4- <i>n</i> -propyl	0.26	~0.8	~3	50	100
5	CH ₂ H 4-isopropyl	0.22	~0.8	~4	50	100
6	CH ₂ H 4-isobutyl	0.21	~0.6	~3	20	100
7	CH ₂ H 4- <i>n</i> -hexyl	0.20	~0.4	~2	50	100
8	CH ₂ H 4-dodecyl	0.28	~0.6	~2	50	100
9	CH ₂ H 4-cyclohexyl	0.33	~0.6	~2	50	100
10	CH ₂ H 4-phenyl	0.17	0.32	1.9	10	70
11	CH ₂ H 4-phenoxy	0.12	0.12	1.0	10	80
12	CH ₂ H 4-(<i>p</i> -chlorophenyl)	0.10	0.12	1.2	5	100
13	CH ₂ H 4-(<i>p</i> -bromophenyl)	0.08	0.10	1.2	2	80
14	CH ₂ H 4-(<i>p</i> -bromophenoxy)	0.07	0.07	1.0	1	40
15	CH ₂ H 4-chloro	0.09	0.14	1.6	5	100
16	CH ₂ H 4-bromo	0.13	0.22	1.7	5	100
17	CH ₂ H 4-benzyl	0.18	~0.3	~2	10	80
18	CH ₂ H 3,4-tetramethylene	> 5.0	—	—	—	—
19	CH ₂ Cl 4-phenyl	0.25	~0.5	~2	—	—
20	CH ₂ CH ₃ 4-phenyl	0.18	~0.4	~2	50	100
21	O H H	> 1.0	> 2.0	—	—	—
22	O H 4-phenyl	0.35	1.5	4.3	—	—
23	O H 4-chloro	0.42	~1.0	~2	—	—
24	O Cl 4-phenyl	0.26	~2.0	~8	—	—
25		0.31	~1.0	~3	20	20

Prothrombin ED₅₀ mg per kg of compound given in three daily doses predicted to increase prothrombin time (as measured by the one stage method of Quick) from a resting value of 16 s to 112 s on the fourth day. Male rats were used throughout. Diphacinone, chlorophacinone, coumatetralyl, S(−) Warfarin and R(+) Warfarin give prothrombin ED₅₀s of 0.22, 0.22, 0.31, 0.30 and 3.3 mg kg⁻¹, respectively in Wistar rats and of ~50.0, >20.0, ~4.4, >50 and >50 mg kg⁻¹ respectively in resistant rats. Resistance factor = ratio of prothrombin ED₅₀s for resistant and normal rats. Diphacinone, chlorophacinone, coumatetralyl, S(−) Warfarin and R(+) Warfarin thus have factors of ~227, >90, ~14, >166 and >15, respectively. Bait trial: rats were exposed to the test baits with no alternative food for 10 d, mortalities were recorded up to 14 d. Female Welsh strain homozygous resistant rats were used throughout. Diphacinone, S(−) and R(+) Warfarin did not cause death at 250 p.p.m. and chlorophacinone and coumatetralyl gave 20% and 40% mortality, respectively, at this concentration. The melting point of compound 1 was 200–201°C, satisfactory analytical data were obtained for the other compounds whose preparation will be described elsewhere. Compounds 3–24 are two component mixtures (thin layer chromatography, TLC) of what are believed to be *cis* and *trans* isomers. In two cases (compounds 10 and 12) the slower running isomer (TLC) was shown to be two to four times as active as the faster running isomer. Since the proportion of the slower running isomer in the mixtures is >50% (TLC) this is unlikely to significantly alter the structure-activity relationships. Patent applications are pending on the 3-(3-*p*-substituted-phenyl-1,2,3,4-tetrahydronaphth-1-yl) 4-hydroxycoumarins.

The substituent in the *para* position of the phenyl group may vary in size with little effect on potency (compounds 4–8) although such limitations are indicated, for example, when the *para* substituent is methyl or tetralyl (compounds 3 and 18), anticoagulant activity is lost. A further aromatic ring joined either directly to the *para* position of the phenyl group (compound 10), or to the *para* position by oxygen or methylene bridges (compounds 11 and 17) seems to be advantageous. The most active compounds contain a *para* halogenophenyl group either joined directly to the *para* position of the phenyl group or joined to this position by an oxygen bridge (compounds 12–14).

A requirement for anticoagulant activity in either strain of rat seems to be a lipophilic group in the *para* position of the phenyl group, suggesting that the substituted phenyl group is providing a point of attachment to a lipophilic site to which Warfarin and other anticoagulants do not bond strongly. The high activity exhibited when the phenyl group contains *para* substituents with somewhat less lipophilic character (compounds 15 and 16) infers that electronic effects may also play some part. Variations in activity, however, do not parallel those reported for the series obtained by replacing the terminal methyl group of Warfarin alcohol by substituted phenyl groups⁴. Substitution in the 7 position of the tetralin ring reduces or has little effect upon activity (compounds 19 and 20). Replacement of the tetralin moiety by chroman or indanyl rings (compounds 21–25) reduces activity and increases resistance. The angle which the phenyl group makes with the indanyl group (compound 25) differs markedly from that made with the tetralin group (compounds 3–20), this may be responsible for the loss of activity.

Bait trials show good agreement with the prothrombin estimations, compounds with the lowest prothrombin ED₅₀ generally being the most active. The indications are that the new materials are well absorbed in the gut and are promising rodenticides. It is interesting to compare kills of resistant rats with those obtained at considerably higher concentrations with the standard anticoagulants (see notes to Table 1).

Tests with two of these compounds (10 and 13) indicate that they act in the same manner as Warfarin (M R H, unpublished) in that they are not classic competitive inhibitors of vitamin K₁. This contrasts with 2-chloro analogue of this vitamin which is also somewhat effective in resistant rats⁶, presumably because it affects a site other than that attacked by Warfarin and is not therefore involved in the resistance mechanism.

One of these compounds (10) has been tested in the field against wild rat infestations resistant to normal anticoagulants, providing control in baits containing only 0.005% (M R H and B D Rennison, unpublished).

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Thermal transitions in collagen and the preferred temperature range of animals

We have shown^{1–3} that the melting point of the molecular collagen of both homeothermic land poikilothermic animals correlates with the upper limit of their range of preferred temperatures (the range of temperatures they will voluntarily tolerate in their natural environment). We now report that for some poikilotherms the lower limit of the range of preferred temperatures also correlates with certain thermal properties of their collagen.

This conclusion was derived from an examination of the mechanical properties of earthworm (*Allolobophora caliginosa*) cuticle and jellyfish (*Aurelia coerulea*) connective tissue when these are heated in physiological saline (0.9% NaCl). Native tissues were used because the properties of collagen are best studied in a condition as close as possible to that in which they exist in nature. Such studies complement and indeed extend work involving purified and soluble collagens.

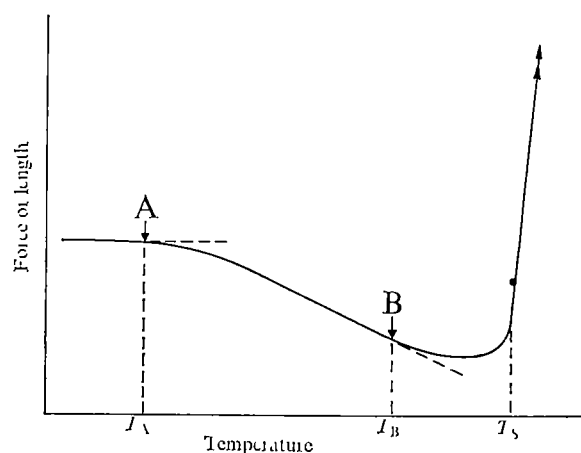
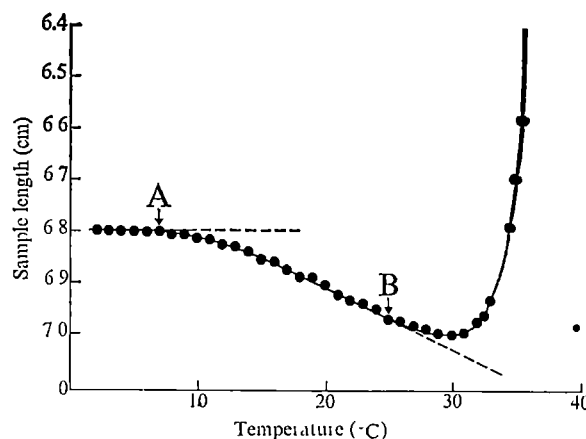


Fig. 1 Diagram defining thermal transitions in a sample immersed in 0.9% NaCl. The ordinate represents the property observed as temperature is increased. The transitions A and B are defined as points of deviation from linearity. T_S is the familiar shrinkage temperature, T_A and T_B are discussed in the text.

The cuticle of the earthworm was removed from an animal which had been killed by holding at 0°C overnight, and this cuticle formed an ideal sample (1–2 μ m thick \times 10 cm long) for direct length measurements as the temperature was varied. The ends of the specimen were clamped together at one end after looping through a glass ring of sufficient mass to just keep the sample straight. Length measurements were made

Fig. 2 A length–temperature curve for a freely suspended sample of the cuticle of earthworm *A. caliginosa* in NaCl. The transitions A and B are indicated by arrows at 7°C and 25°C, respectively. T_S takes place at \sim 35°C.



with a cathetometer. As the applied stress was virtually zero, this experiment amounts to one-dimensional dilatometry.

The jellyfish samples were cut from the bell of the animal and stored in an acetone-water mixture until required. They were then washed and soaked in 0.9% NaCl overnight, by which time they had the appearance of fresh material. In the case of jellyfish samples it was not convenient to measure length, and the thermal behaviour was determined by observations of stress relaxation in the slightly extended specimen. One end of the specimen was held in a fixed clamp while the other was connected to a very stiff force transducer. Stress variation was recorded on a chart recorder. Ultimately, both methods detect molecular relaxation processes whether they be first or second order thermal transitions. The stress relaxation method, however, is somewhat more versatile as it is independent of sample dimensions.

Before beginning an experiment the samples were conditioned at the lowest experimental temperature for at least 16 h, or until, in the case of stress relaxation experiments, equilibrium was attained. Two heating rates were used: 0.1 or 0.5°C min⁻¹, but no difference could be detected between results obtained with either.

Table 1 Mean values for the three thermal transitions defined in Fig. 1 for the collagen of two earthworms and a jellyfish

Animal	No. of experiments	T_A	T_B	T_S	Preferred temperature range
<i>A. caliginosa</i>	10	8	23	35	10–23 (ref. 10)
<i>E. foetida</i>	6	12	23	35	16–23 (ref. 10)
<i>A. coerulea</i>	7	17	27	50	17–25 (ref. 3)

The range of preferred temperatures for the two earthworms reported by Grant¹⁰ are listed. In the case of the jellyfish, the range has been listed as that over which the pulsation rate of the bell is uniform and linear³, but is also close to the seawater temperature limits which the animal frequents.

Both experiments gave rise to a characteristic curve (Fig. 1). There is always a linear region in which there is little change in length or force with temperature. The first transition occurs at A, then a less marked transition at B, and finally the sample shrinks. The temperatures at which the three transitions take place are denoted by T_A , T_B and T_S . At T_S , the familiar shrinkage temperature, the length decreases for the freely suspended sample and force increases in the stress relaxation experiment, because the sample is constrained. As long as the temperature is not allowed to exceed T_B by more than 2–3°C the sample may be cooled to below T_A and the cycle repeated. In other words the transitions are reversible. In Figs 2 and 3 the dotted lines are drawn to show more clearly the transition at A and B. The mean values for the transitions in all experiments, including another species of earthworm *Eisenia foetida* are given in Table 1.

A full description and interpretation of these transitions will be given elsewhere. Here we are interested only in the biological significance of the transitions, especially those occurring at A and B. We have already said that T_S marks the region of the well known thermal shrinkage phenomenon, which has been shown to be a manifestation of the melting of crystalline collagen^{4,5}.

The mean temperature of the transition at B of the collagen of *A. caliginosa*,^{1,6} and *A. coerulea*³ agrees with the temperature at which the molecular collagen melts in dilute solution (that is, T_D , the mid-point of the transition as determined by optical rotation or viscometry), or what we have shown to be equivalent—the temperature at which these tissues shrink when heated in HCl solution at pH 1 (ref. 7). Determination of T_D for the collagen of *E. foetida* in HCl gave 22°C, which again is close to T_B . A number of other earthworms^{1,6} have a T_D value of

22°C, and Maser and Rice⁸ and Josse and Harrington⁹ using optical rotation report 22°C for another earthworm *Lumbricus terrestris*. So, we think it justifiable to equate the tissue transition T_B with T_D , the molecular transition.

Before commenting on the transition at A we need to consider the following. The molecular melting temperature, T_D , of the collagen of a wide range of animals agrees with the upper limit of their preferred thermal range, if they are poikilothermic, or with their normal body temperature if they are homeothermic^{1–3}. Now, in view of the preceding paragraph, when dealing with tissues, we can say that T_B correlates in the same way. When we found the transition at A, we wondered if it had any relation to the lower limit of the preferendum. This turned out to be the case on examining the literature. First, our own data³ for the jellyfish *A. coerulea* showed that, over the range 17°–26°C the pulsation rate of the bell of a live animal was uniform and linear with temperature, at 15°C the jellyfish had become sluggish, while above 28°C the pulsation rate dropped very rapidly. Furthermore, the mean limits of surface water temperature along the New South Wales coast where the animal was taken, are 17°–23°C (ref. 3).

Although we originally linked the upper value of 26°C (as determined by the pulsation behaviour of the bell) with the value of the melting temperature of its collagen, we did not look for evidence of a structural transition near the lower limit of 17°C. These data have been incorporated into Fig. 3, and an examination of the figure shows that thermal transitions A and B (curve 1) are very close to the lower and upper limits of the normal pulsation rates of the jellyfish respectively.

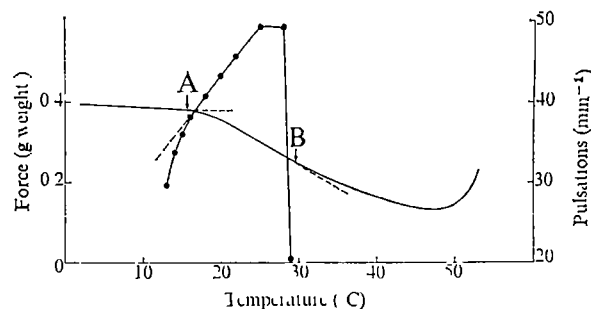


Fig. 3 A stress relaxation-temperature curve for jellyfish connective tissue in 0.9% NaCl, together with data (●) showing the pulsation rate of the bell plotted against temperature for a live jellyfish in seawater³. The species was *A. coerulea*.

(curve 2), and both points correspond well with the limits of its range of preferred temperatures³. Second, in the case of the earthworm it has been reported by Grant¹⁰, that *A. caliginosa* and *E. foetida* have thermal preference ranges of 10°–23°C and 16°–23°C respectively. The upper limits agree very well with T_B in Table 1, and the lower limits are close to T_A for each species.

We suggest that transition temperature T_A marks the lower limit of the animal's range of preferred temperatures just as T_B (or, T_D in dilute solution studies on molecular collagen) marks the upper limit. Further, it seems that transition A represents the beginning of a disorganisation of molecular collagen, but that complete melting of the molecule in dilute solution will not take place until a temperature greater than T_B is reached. Whether the properties of collagen determine the range of preferred temperatures or vice versa, is an open question.

When we compare dilute solution studies with tissue studies only T_D and T_B are coincident. There is no evidence in the work of either Maser and Rice⁸ or of Josse and Harrington⁹ of a transition at a temperature corresponding to T_A . This may be because they used a different species of earthworm, or because the use of native tissue preserves non-collagenous components

which give rise to the transition at A, or possibly, there are other collagens with lower thermal stabilities, which have been destroyed by the usual solubilisation procedure

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Reduced maximal levels of derepression of the isoleucine-valine and leucine enzymes in *hisT* mutants of *Salmonella typhimurium*

STRAINS of *Salmonella typhimurium* with a mutation in the *hisT* locus, produce an enzyme that cannot convert two uridine residues to pseudouridine in the anti-codon region of tRNA^{His} (refs 1 and 2). This mutation allows constitutive synthesis of the histidine biosynthetic enzymes^{1,3}. Recently, *hisT* mutants were found to have changes in tRNA^{Leu} (refs 1, 4 and 5), in tRNA^{Ile} (ref 6), and to be partially derepressed for the isoleucine-valine biosynthetic enzymes^{5,6}. This suggests strongly that tRNA is part of the repression machinery for these enzymes. We report here that *hisT* strains are greatly limited in their ability to derepress the isoleucine-valine and leucine enzymes during leucine starvation. Thus, the *hisT* mutation alters regulation of these enzymes in two ways, it seems to cause a marked reduction in their maximal level as well as leading to a partial loss of repressibility.

A leucine-requiring strain of *S. typhimurium* containing the *hisT1504* lesion was constructed by transduction with phage P22. When the strain was grown in the presence of excess branched-chain amino acids, the level of the isoleucine valine and leucine enzymes was essentially the same as that found for the prototrophic *hisT1504* parent strain (Table 1). During leucine starvation these enzymes were increased two- to three-fold in the strain containing both *hisT* and *leu* mutations, while the isogenic strain without the *hisT* mutation was derepressed 14- to 116-fold when grown under the same conditions (Table 1). This effect seems specific for the isoleucine-valine and leucine enzymes since the levels of two unrelated enzymes, hexokinase and acetylornithinase, are equivalent in both strains during leucine restriction (M F unpublished).

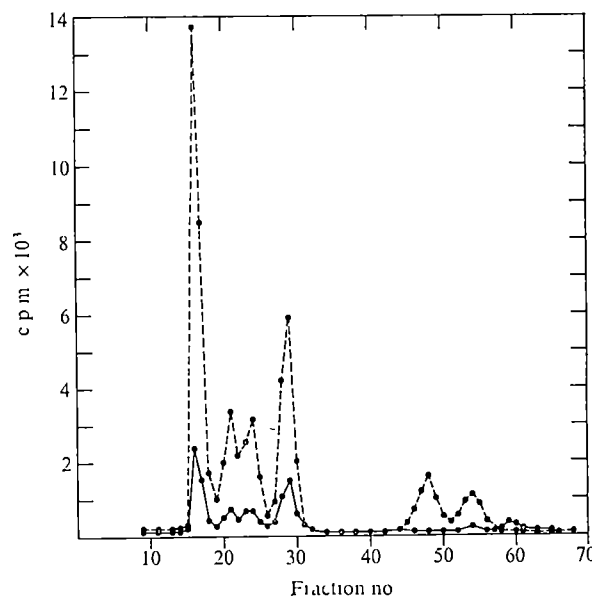


Fig. 1 *In vivo* levels of charged tRNA^{Leu} in the *hisT* strain grown on limiting leucine. The cells were grown in minimal medium with L-isoleucine (50 µg ml⁻¹), L-valine (100 µg ml⁻¹) and L-leucine (9 µg ml⁻¹). The tRNA was isolated as described previously⁵ except that after phenol extraction and centrifugation, the aqueous phase was layered on to a DEAE-cellulose column equilibrated with 0.3 M LiCl in 0.1 M sodium acetate buffer, pH 5.0. The column was then washed with several bed volumes of this buffer. The tRNA was eluted with 1.0 M LiCl and precipitated with 2.5 volumes of ice-cold ethanol. The isolated tRNA was divided into two portions, one was kept as control and the second sample was oxidised with sodium periodate as described previously⁵. The control and periodated samples were charged with ³H-leucine and ¹⁴C-leucine respectively and mixed and cochromatographed on an RPC-5 reversed-phase column as described previously⁵. The altered mobility of the last two isoacceptor species from the periodated sample is an artefact of the periodate oxidation procedure⁵ (A A R and M F, unpublished). ● — ●, ³H unperiodated, ● — ●, ¹⁴C periodated.

To investigate this further, both strains were grown with various amounts of leucine. The levels of the isoleucine-valine and leucine enzymes in the strain with the *hisT* mutation did not change more than threefold for the entire range of leucine concentrations (Table 2). There was a strong inverse relationship between the amount of leucine and the enzyme levels found in the isogenic strain. Growth of these strains in a chemostat with a limiting supply of leucine produced similar results (Table 2). Under all conditions tested the *hisT* mutation seems to have curtailed the range of derepression during leucine limitation as well as reducing the maximal level of the isoleucine-valine and leucine enzymes that can be made under these conditions.

The limited ability of strains with a *hisT* mutation to derepress is not common to all strains that show partial constitutive

Table 1 Effect of the *hisT* mutation on derepression of the isoleucine-valine and leucine enzymes and levels of charged tRNA

Mutation	Growth conditions	Threonine deaminase	Specific activity		tRNA charged <i>in vivo</i> (%)	
			Acetohydroxy acid synthetase	β-IPMD	Leucine	Valine
<i>leu124</i>	Excess leucine	8.2	0.7	1.7	82	90
<i>leu124</i>	Limiting leucine	107.6	82.3	91.2	27	87
<i>hisT1504 leu124</i>	Excess leucine	21.2	7.6	12.4	91	88
<i>hisT1504 leu124</i>	Limiting leucine	33.6	18.9	20.3	18	92

All bacterial strains were derived from *S. typhimurium* LT-2. The strain containing the *hisT1504* mutations was constructed by P22 transduction⁷. The *hisT* allele was transduced into a strain containing the mutations *aroD38 leu124*, selecting for Aro⁺ recombinants. The *hisT* lesion was identified by colony morphology⁸, growth rate⁹, tRNA^{Leu} profile^{1,5} and enzyme levels^{5,6}. The minimal medium and growth conditions were as described previously⁵. The minimal medium was supplemented with L-isoleucine (50 µg ml⁻¹), L-valine (100 µg ml⁻¹) and excess L-leucine (50 µg ml⁻¹) or limiting L-leucine (7.5 µg ml⁻¹). Enzyme specific activity is expressed as µmol of product per mg of protein per hour. The preparation of the crude cell extracts and the measurement of enzyme activity and protein was as described previously⁵. The leucine enzyme measured in this study was β-isopropylmalate dehydrogenase (β-IPMD). Isolation and periodate oxidation and the percentage of tRNA that existed in the charged form *in vivo* were as described previously⁵.

Table 2 Effect of the *hisT* mutation on derepression during growth limitation on various concentrations of leucine

Mutation*	L-Leucine ($\mu\text{g ml}^{-1}$)	Specific activity		β -IPMD
		Threonine deaminase	Acetohydroxy acid synthetase	
<i>leu124</i>	7.5 chemostat†	123.2	82.5	61.6
<i>leu124</i>	7.5	101.1	61.2	97.4
<i>leu124</i>	12.5	59.9	45.0	76.3
<i>leu124</i>	15	53.3	19.4	47.0
<i>leu124</i>	17.5	42.3	16.0	32.8
<i>leu124</i>	20	27.7	8.3	18.1
<i>leu124</i>	50	5.6	1.0	1.4
<i>hisTleu124</i>	7.5 chemostat	51.0	25.0	28.0
<i>hisTleu124</i>	7.5	41.0	26.1	23.0
<i>hisTleu124</i>	12.5	30.0	10.8	14.8
<i>hisTleu124</i>	15	28.5	10.8	13.2
<i>hisTleu124</i>	17.5	29.6	10.6	12.3
<i>hisTleu124</i>	20	27.4	7.9	12.0
<i>f1rB1leu124</i>	7.5	138.0	118.6	130.0
<i>f1rB1leu124</i>	50	63.8	77.0	55.2

*The strain containing the *f1rB1leu124* mutations was constructed by transducing the *leu124* mutation into a strain containing the mutations *ara1r123*, selecting for *Ara*⁺ recombinants. *f1rB* strains are resistant to the leucine analogue 5',5'-trifluoroleucine and are constitutive for enzymes forming branched-chain amino acids. The biochemical basis for this phenotype is unknown¹⁰.

†In experiments with a chemostat the minimal medium was supplemented with L-isoleucine (50 $\mu\text{g ml}^{-1}$), L-valine (100 $\mu\text{g ml}^{-1}$) and L-leucine (7.5 $\mu\text{g ml}^{-1}$). Enzymes were measured after four doublings. All other experiments involved Erlenmeyer flasks for measurements of growth and enzyme levels. The minimal medium contained L-isoleucine, L-valine and L-leucine, as indicated. In all experiments the doubling time of the *hisT* mutant was 30–35% less than the isogenic strain. This reduced growth rate is about the same as reported for *hisT* strains prototrophic for leucine, grown in minimal medium¹. For all other conditions and explanations see Table 1.

synthesis of the enzymes. A strain with a *f1rB1* mutation is highly rederepressed for the isoleucine–valine and leucine enzymes due to a mutation other than *hisT* (ref. 10). We transduced the same *leu* mutation into this strain as we had for the *hisT1504* mutant. Derepression of the isoleucine–valine and leucine enzymes during leucine starvation was greater in this mutant than in the isogenic leucine-requiring strain and three to five times greater than for the *hisT* leucine auxotroph (Table 2).

A direct correlation has been found between decreased levels of charged tRNA^{Leu} and derepression of the isoleucine–valine and leucine enzymes¹¹. The difference in derepression found in the strain with the *hisT* mutation might therefore be due to variations in tRNA^{Leu} acylation during leucine starvation. This does not seem to be the case since bulk tRNA^{Leu} was about equally deacylated in both strains when they were starved for leucine (Table 1). In addition, the *in vivo* charging of the individual species of tRNA^{Leu} was examined by the reversed-phase chromatography system (RPC-5) of Kelmers and Heatherly¹². All components of leucine-acceptor activity were highly deacylated when the strain with the *hisT* lesion was grown on limiting amounts of leucine (Fig. 1).

Previous work has established that tRNA^{Leu} is altered in *hisT* mutants and that these strains are partially derepressed for the isoleucine–valine and leucine enzymes^{1,5,6}. In addition, our results suggest that the *hisT* mutation alters further the repression mechanism in these pathways by reducing their maximal expression during leucine limitation. These data offer additional evidence for the involvement of tRNA^{Leu} in repression of the isoleucine–valine and leucine enzymes. The dual effect of the *hisT* mutation on the regulation of these enzymes suggests that the lack of pseudouridine in the anticodon region of tRNA^{Leu} introduces a conformational change in the tRNA which affects both minimal and enzyme levels. Thus, the conformational change which may normally occur in tRNA^{Leu} during aminoacylation to cause repression may be defective in tRNA^{Leu} from *hisT* mutants. In addition, this conformation may elicit partial repression even if the tRNA is deacylated. Alternatively, a species of tRNA^{Leu} may have a positive role in regulation. The alteration of such a species in *hisT* mutants could prevent

the full expression of the isoleucine–valine and leucine enzymes. Because of the pleiotropic nature of the *hisT* mutation^{1,2}, it is possible that another altered tRNA rather than, or in addition to tRNA^{Leu} is responsible for these changes in repression. Cortese *et al.*⁶ reported a possible alteration in a minor species of tRNA^{His} in *hisT* mutants. Changes in tRNA^{His} do not, however, seem to alter the regulation in these systems since the form of acetohydroxy acid synthetase that is not subject to feedback inhibition by valine is not disproportionately elevated in *hisT* strains (unpublished observations of M. F.). It has been shown that due to an alteration in the isoleucine 'repression signal' the resistant but not the valine-sensitive form of acetohydroxy acid synthetase is derepressed^{13,14}. It is also conceivable that the encoded product of the *hisT* gene could be involved in regulation of the isoleucine–valine and leucine enzymes. Utilisation of *hisT* mutants in an *in vitro* protein synthesising system will aid the choice from among these possibilities.

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Chromosome-21-dosage effect on inducibility of anti-viral gene(s)

UNLIKE the bacterial situation little is known about gene regulation in mammalian cells. Inducible systems are being used to study regulatory gene functions in normal and abnormal mammalian cells^{1–3}, and have shown for example, that inhibitors of macromolecular synthesis enhance rather than inhibit the expression of an inducible enzyme. Actinomycin D enhances the induction of tyrosine aminotransferase (TAT) by steroid hormones in hepatoma cells⁴. Commonly referred to as superinduction, this effect has led to the hypothesis that a regulatory gene(s) modulates the expression of the structural TAT gene⁴. Of course, alternative explanations have been offered for the superinducing effect of actinomycin D (refs 5 and 6). Similarly, the induction of interferon by viruses and poly(I).poly(C) and the induction of the anti-viral state (AVS) by interferon have been used to probe regulatory mechanisms in normal mammalian cells^{7–13}. It has been demonstrated that with judicious use of metabolic inhibitors the amount of interferon and the level of AVS induced can be enhanced 100–1,000 times and 10–100 times respectively, over that obtained in cultures exposed to inducer alone. This suggested that one or more regulatory gene(s) modulate the expression of the structural gene for interferon and AVS^{7–19}.

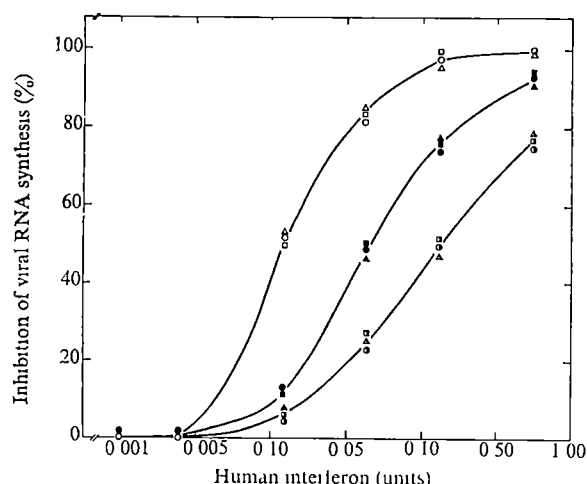


Fig. 1 Induction of the AVS in human skin fibroblasts: monosomic (cell line 198 halved symbols), disomic (cell line 141 closed symbols), and trisomic (cell line 258, open symbols) for chromosome 21 by human interferon. Three sets of experiments were performed for each cell line described.

I have used a new approach to assess the presence of a regulatory gene function which modulates the expression of the structural gene for AVS. I found evidence to suggest that the regulation of the anti-viral genes in human cells is controlled by regulatory gene element(s) located on a separate chromosome.

AVS was induced in human fibroblast cultures trisomic (T-21) disomic (D-21) and monosomic (M-21) for chromosome 21, trisomic for chromosome 18 (T-18), monosomic for chromosome 5 (M-5) and carrying a partial deletion of chromosome 4 (M-4) by exposing them to various concentrations of human interferon. The level of AVS induced was estimated by assessing the amount of interferon required to inhibit viral RNA synthesis by 50%. Figure 1 and Table 1 show that M-21 fibroblasts were less sensitive to the anti-viral action of interferon than normal diploid fibroblasts, which were in turn less sensitive than T-21 fibroblasts. The concentration of interferon required to induce AVS in M-4, M-5 and T-18 fibroblasts falls within the same range of concentrations required to induce AVS in normal diploid fibroblasts, suggesting that the effect of

Fig. 2 Gene dosage effect of chromosome 21 on the induction of the AVS in human fibroblasts monosomic, disomic and trisomic for chromosome 21. Each point represents the mean reciprocal concentration of human interferon (HuIF) required to induce the AVS in the fibroblasts. The mean concentration of interferon is derived from the figures given in Table 1.

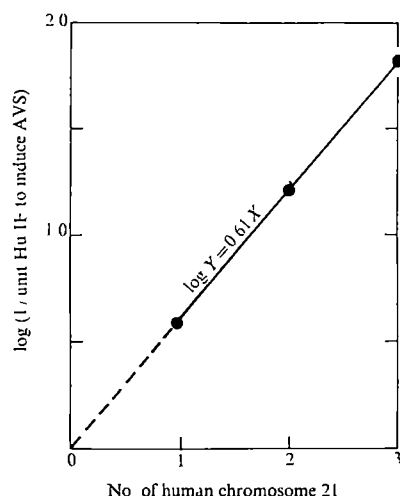


Table 1 Induction of AVS in human skin fibroblasts

Cell type	Identification	Units human interferon inhibiting vital RNA synthesis by 50%
Monosomic 21	no	
	GM-137	0.24, 0.24, 0.27
	GM-230	0.26, 0.29
Disomic 21	198	0.30, 0.24, 0.25, 0.24
	GM-37	0.060, 0.064
	GM-181	0.055, 0.061
Trisomic 21	141	0.062, 0.064, 0.059, 0.069
	GM-258	0.0157, 0.0157, 0.0156, 0.0180
	DS-GG1	0.0157, 0.0172
Partially deleted 5	DS-GG2	0.0160, 0.0157
	GM-72	0.060, 0.065
	GM-71	0.062, 0.059, 0.060
Trisomic 18	(Ref. 21)	0.059, 0.070

Human fibroblast cultures established from skin biopsies were provided by the Mammalian Genetic Mutant Cell Repository, Camden, New Jersey, the Repository for Mutant Human Cell strains, Montreal and by Drs J. Mahoney and K. Halloran of Yale University. Three human fibroblast cultures trisomic for chromosome 21 (T-21) designated GM-258, DS-GG1 and DS-GG2, three human cultures monosomic for chromosome 21 (M-21) designated GM-137, GM-230 and 198, three normal diploid human skin fibroblasts, disomic for chromosome 21 (D-21) designated GM-37, GM-181 and 141, and two human fibroblast cultures, one known to carry a partially deleted chromosome 4 and the other known to have one less chromosome 5 designated GM-72 and GM-71 respectively were used. A trisomic 18 human fibroblast (T-18) derived from a previous study²⁷ was also used as one of the controls for the trisomic 21 cultures. The monosomic 21 lines used were verified by the Mammalian Genetic Mutant Cell Repository to contain 45 human chromosomes and to lack one chromosome 21. No translocation was evident. The amount of vesicular stomatitis virus replication was assayed by Skehan's method²⁸ except that (a) the cultures were exposed to actinomycin D ($4 \mu\text{g ml}^{-1}$) for 1 h before the viral challenge and (b) the cultures were incubated with the virus for 8 h instead of 14–17 h at 37°C . This modification of the procedure was introduced to reduce variability in the estimation of viral RNA synthesis. The results listed for the same cell type were derived from the fibroblasts at early cell passages. The interferon used in this study was derived from a large batch of human interferon that was contributed by Dr J. Valenta of Smith, Kline and French Pharmaceutical. This was purified through two cycles of gel filtration on Sephadex G-75 columns. The purified interferon had a specific activity of $\sim 1 \times 10^5 \text{ U mg}^{-1}$ protein. The unit of interferon described here is based on the internal standard of Smith, Kline and French interferon.

chromosome 21 on AVS is not a generalised effect resulting from the loss or gain of a human chromosome.

Figure 1 and Table 1 also show that the inducibility of the AVS in human cells increases nonlinearly for each increment in the number of human chromosome 21 present. To analyse this effect, the mean concentration of human interferon required to induce the AVS in fibroblasts containing one, two or three chromosome 21 (Table 1) was plotted logarithmically against the number of chromosome 21. A straight line was obtained which can be expressed by the equation

$$\log Y = 0.61X$$

where Y denotes the inducibility of AVS and X the number of chromosome 21 (Fig. 2). Thus, the inducibility of AVS increases logarithmically as the number of chromosome 21 increases linearly. Extrapolation of the curve to the origin suggests that a cell cannot be induced for AVS if chromosome 21 is absent. This confirms the assignment of the AVS gene(s) to chromosome 21 (ref. 18).

Before attempting to interpret the significance of this logarithmic gene dosage effect, it is useful to consider events that might contribute to the establishment of the AVS. They are (1) the uptake of interferon by its receptor sites, (2) the depression of the structural gene(s) which code(s) for AVS by an inducer, presumably interferon, (3) the transformation of cells into an AVS through production of an anti-viral protein, and (4) the modulation of the level of the AVS by regulatory gene element(s).

The existence of interferon receptor sites has not been substantiated. The existence of the structural gene(s) described in step (2) is based on genetic analysis of somatic cell hybrids derived from hamster \times mouse, monkey \times mouse

and human×mouse¹⁷⁻²⁰ This has led to the assignment of the gene(s) which codes for AVS and the cytoplasmic form of superoxide dismutase to chromosome 21 in man¹⁸

The mechanisms of transformation of a cell to an AVS in step (3) is unclear, though it is presumed to be mediated by a protein²¹ which inhibits the translation of exogenous mRNA²²⁻²⁶ Conceivably the chromosome-21-directed AVS gene(s) produces this inhibitor substance I have already discussed the existence of the regulatory gene function in step (4)

Accordingly, any increase in the number of chromosome 21, should be followed by a linear and proportional increase in the amount of chromosome-21-directed gene products Instead my data show a logarithmic increase, showing that other complex mechanisms, presumably of a regulatory nature are involved One such mechanism is that (1) the regulatory gene element(s) postulated by Chany *et al*¹⁰ or (2) the repressor substances which normally maintain the anti-viral gene(s) in a non-constitutive state, is not located on chromosome 21 The effect of an increase in chromosome 21 would be an imbalance in the ratio of the AVS gene(s) and its regulator gene element(s) which is postulated to be located on a chromosome other than 21 This imbalance could mitigate the normal role of the regulator gene(s) in controlling the levels of AVS directed by the AVS gene(s)

The logarithmic increase in the amount of chromosome-21-directed product as the number of chromosome 21 increases linearly indicates that cellular induction of AVS can be mediated through interchromosomal gene interactions This can be tested, for example, using an interferon selective system on a population of aneuploid human cells to select for cells expressing high and low levels of AVS The levels of AVS in turn are determined by chromosome 21 dosage and dosage of the chromosome(s) other than chromosome 21 carrying the regulator gene element(s) for AVS In any event, it is possible that cellular regulation by interchromosomal gene interaction is responsible for human disorders associated with the loss or gain of a single human chromosome

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Multiple conformations of ribosomal precursor RNA

We wish to propose an explanation for the heterogeneity shown¹ by ribosomal precursor RNAs on gel electrophoresis The clearest demonstration of this phenomenon is shown in Fig 1 ³²P-labelled RNA was eluted from single gel slices taken across the peak, and rerun in the presence of ³H-labelled marker The ³²P-RNA samples run in their original positions and not coincidentally with the marker, that is, the width of the peak does not result from diffusion alone

A heterogeneity of the 45S precursor of L cells has also

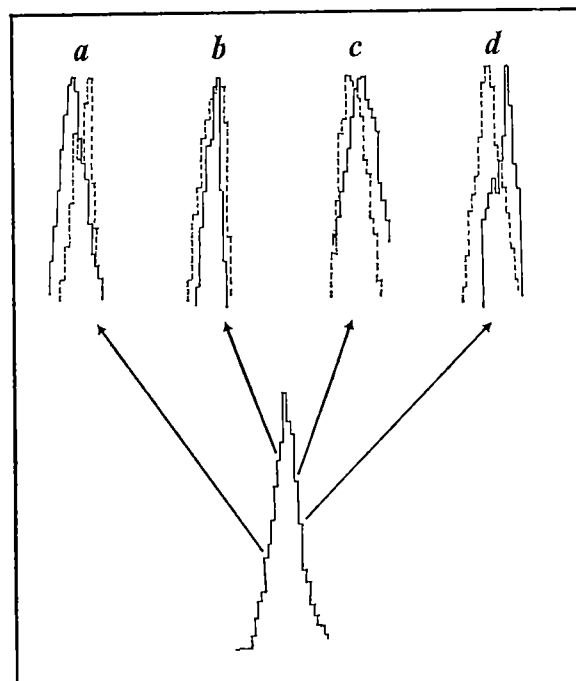


Fig 1 The heterogeneity effect The lower histogram represents ³²P-radioactivity in 0.5 mm slices from the 40S peak of *Xenopus* RNA run on a 2.4% polyacrylamide gel RNA was eluted from individual gel slices by the method described¹ Each sample was mixed with 40S RNA labelled with ³H-uridine and rerun on a second 2.4% gel (upper histograms a-d) These gels were sliced to 0.5 mm and the slices were counted in scintillator ---, ³H-Radioactivity, —, ³²P-radioactivity The methods used to label the cells, extract the RNA, run, slice and count the gels have been described^{2,3}

been reported, which was demonstrated by the effect of the radioactive pulse length on the gel mobility⁴ Although mobility in polyacrylamide gels depends mainly on molecular weight, it is known that other factors can have an effect⁵ Three possible explanations for the phenomenon may be proposed, although they are not necessarily mutually exclusive (1) The original RNA peak is actually a mixture of components differing in length, perhaps because of processing reactions, (2) heterogeneity in primary sequence, because of divergence between the multiple ribosomal gene copies, is sufficient to cause a heterogeneity in gel mobility, and (3) the heterogeneity is caused by differences in secondary structure between otherwise identical molecules

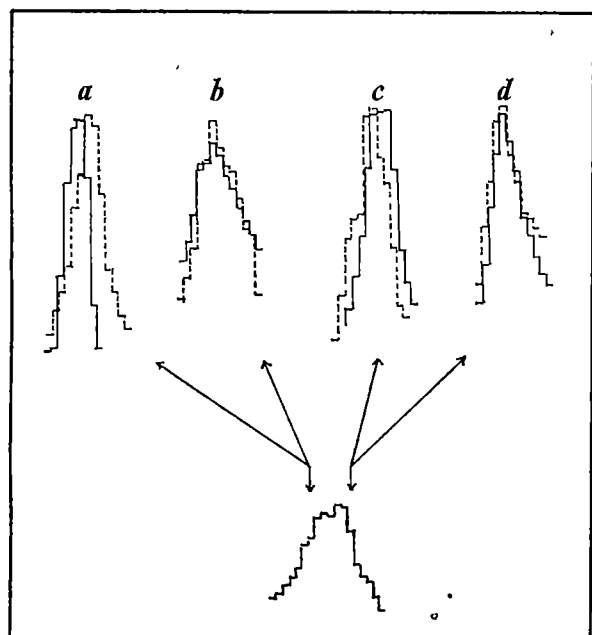
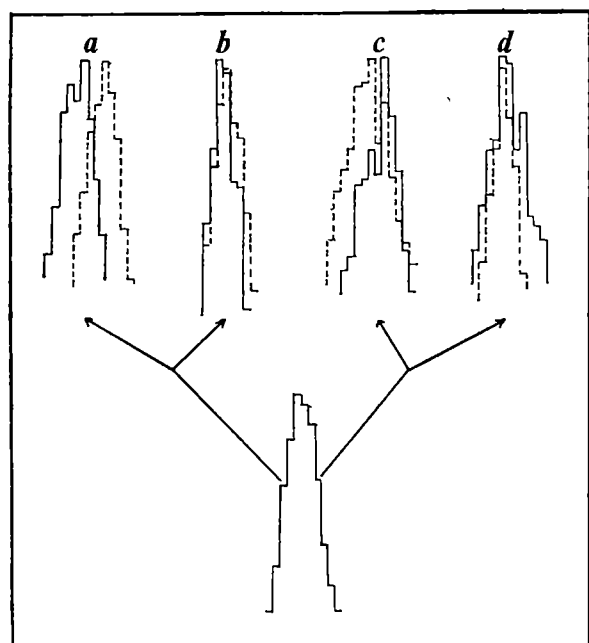


Fig. 2 Abolition of heterogeneity by formamide treatment of *Xenopus* 40S RNA. ^{32}P -labelled RNA from the indicated slices was mixed in E buffer³ with total cell RNA pulse labelled with ^3H -uridine. The mixtures were divided, half being run as controls (a and c) and the other half (b and d) being treated before running with 70% formamide at 37° C for 5 min. The formamide was deionised by passage through mixed Amberlites IR 120/IRA-400. All glassware and solutions were sterilised with a dilute solution of diethyl pyrocarbonate. -----, ^3H -Radioactivity, ———, ^{32}P -radioactivity.

Figures 2 and 3 show the effect of partial denaturation of the eluted RNA fraction (plus marker) using heat treatment or formamide. Figure 2 shows the 40S *Xenopus* precursor^{3,6} and Fig 3 the 42S precursor from yeast^{7,8}, in our case *Schizosaccharomyces pombe*. In both experiments the heterogeneity has been abolished by the treatment, thus ruling out the first of the possible explanations. In addition,

Fig. 3 Abolition of heterogeneity by heat treatment of yeast 42S RNA. The experimental design was the same as that of Fig 2 except that the treatment consisted of heating to 60° C for 5 min. -----, ^3H -Radioactivity, ———, ^{32}P -radioactivity.



the 40S RNA has a unique 5' terminal nucleotide⁹. The second explanation could still be correct insofar as any primary sequence divergence might manifest itself as secondary structure differences. If it could be shown that the RNA reanneals during the period for which the gel is run, usually 4 h, this would be unlikely. It should be stressed that the formamide treatment lasts for 5 min only and the gels are normal ones, run in an aqueous medium rather than the denaturing formamide medium described by Staynoff *et al*¹⁰.

The total RNA preparation was treated with formamide and the RNA from single slices of the precursor peak rerun with marker. The heterogeneity is evident from Fig 4, indicating that the RNA does reanneal within the gel. We would not, therefore, expect the experimental procedure of Figs 2 and 3 to remove heterogeneity caused by the effect of primary structure divergence on secondary structure. Furthermore, since heterogeneity can reappear after treatment of the total RNA, it would seem that the relevant secondary structure differences are characteristic of the molecules themselves rather than of other influences *in vivo*. For example, ribosomal proteins can affect the conformation adopted by ribosomal RNA¹¹.

The most likely interpretation of these experiments is that several secondary structure configurations are accessible to the molecules but that they do not readily interconvert once formed. Unless there is something peculiar

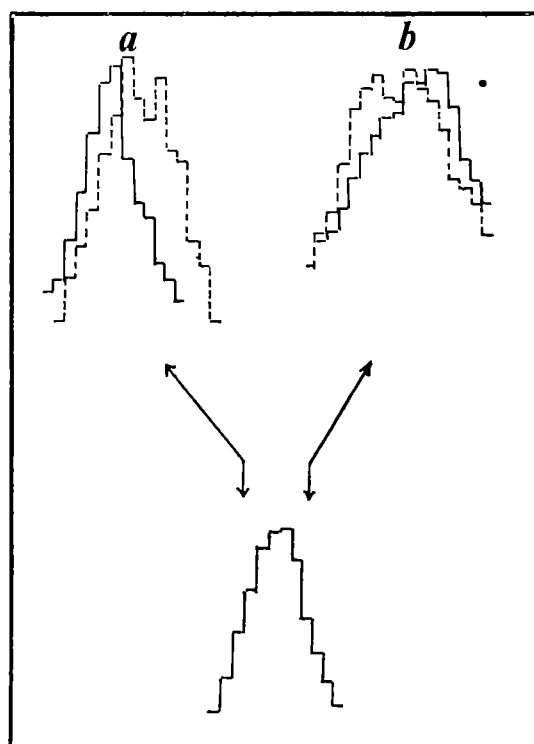


Fig 4 Retention of heterogeneity after formamide treatment of the ^{32}P -labelled total cell RNA before running it on the first gel. -----, ^3H -Radioactivity, ———, ^{32}P -radioactivity.

about ribosomal precursor RNAs this suggests that other high molecular weight RNAs should show the same effect, and in fact we have found a small heterogeneity *Xenopus* 28S RNA and yeast 18S RNA which is not found after partial denaturation.

An RNA molecule is usually presumed to have a definite secondary structure which is the most stable arrangement of its primary sequence in antiparallel A-U and C-G base paired loops^{12,13}. The most stable arrangement may not be the same in different chemical environments, for

example, denatured forms of transfer and 5S RNA are known which are produced by the action of urea and EDTA^{14,15}, and in the case of 5S RNA the two forms are known to have different arrangements of the base paired regions¹⁶.

What our experiments suggest is that a collection of identical molecules placed in the same environment can spontaneously attain a range of different secondary structures. Structures may be regarded as different when the activation energy for interconversion is too high for rapid equilibration to occur (by comparison the activation energy for 5S renaturation has been found to be about 65 kcal/mol¹⁷). We would expect this property to be more evident for the high molecular weight RNAs because the greater length of the sequence should allow more base pairing arrangements, and indeed the low molecular weight RNAs isolated with modern techniques seem to be homogeneous in their three-dimensional structure, as evidenced, for example, by the crystallisation of tRNA^{18,19}.

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Crystal structure of proflavine, a DNA binding agent

THE subject of DNA-small molecule binding has received much attention in recent years. Particular emphasis has been placed on aminoacridine binding¹, and many studies have employed proflavine (Fig 1) as perhaps the classical interacting agent of these series of drugs. The DNA-binding properties of proflavine cause it to act as a frameshift mutagen, and this ability has been extensively utilised in molecular genetics².

Proflavine has been shown to bind to DNA in two ways^{1,3}. First a strong interaction at low drug concentrations, involving intercalation of the planar drug molecule between successive base pairs⁴, and second a weaker process at higher drug levels primarily concerned with interactions with the phosphates external to the DNA double helix, although the precise stereochemical details of both the binding modes are not clear. It has been suggested, in a modification to the original intercalation model, that strong binding also involves ring

nitrogen-phosphate interactions⁵. As part of our studies directed to understanding drug-DNA binding, we have determined by single-crystal X-ray analysis the structure of proflavine itself, as the biologically active hemi-sulphate.

Proflavine hemi-sulphate crystallises from aqueous solution as well-formed, deep-red elongated prisms, belonging to the monoclinic space group $P2_1/c$. The cell dimensions are $a=12\ 703(1)$, $b=19\ 940(2)$, $c=21\ 487(2)$ Å, and $\beta=92\ 24(1)^\circ$. This surprisingly large unit cell, together with measurements of crystal density, suggested that there were several proflavine

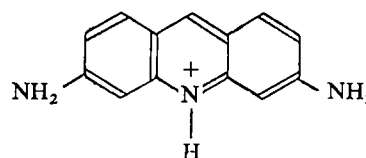


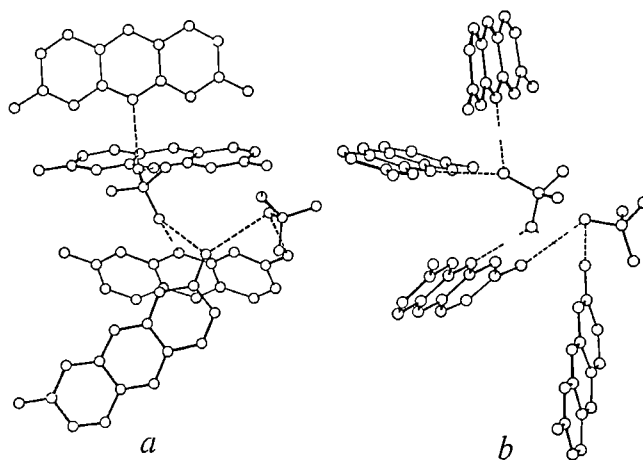
Fig. 1 Proflavine

molecules in the crystallographic asymmetric unit, and this has been confirmed by our structure analysis. A total of 5,600 independent reflection intensities were measured on an automatic four-circle diffractometer.

The structure was solved by a combination of direct methods⁶ and reciprocal-space search procedures⁷, and refined by least-squares methods to a final discrepancy index of 0.0736. (The full details of the structure solution, as well as other crystallographic discussion, will be published elsewhere.) All the hydrogen atoms were located, except those attached to water molecules. As suggested by Albert⁸, the cationic charge of the proflavine rings has been found to reside exclusively on the central nitrogen atom, as evidenced by the location of a hydrogen atom attached to this atom, and coplanar with the ring. Our analysis has revealed that the asymmetric unit consists of four proflavine molecules, together with two sulphate ions and seven water molecules, a total of 81 independent non-hydrogen atoms. These four proflavine molecules are not related by any local symmetry elements.

The crystal structure has a number of features of interest. As might be expected, the lattice is held together by a complex network of hydrogen-bonding and electrostatic interactions. Figure 2 shows one unit of four proflavines, and illustrates their peculiar clustering around the sulphate ions, with one of

Fig. 2 a and b two views of the four proflavine molecules and two sulphate ions in the crystallographic asymmetric unit. Dashed lines represent intramolecular interactions between these, other interactions with neighbouring molecules, and water molecules, have been omitted for reasons of clarity. The right-hand view represents a 90° rotation about the vertical axis, with respect to the left-hand one.



the latter being particularly intimately involved. Three of the four proflavines are strongly hydrogen—(and electrostatically) bonded from their central ring nitrogen atoms, to this sulphate. The fourth molecule is attached by an amino nitrogen, to both sulphate ions. The charged nitrogen to sulphate-oxygen interactions are strong ones, with nitrogen-oxygen distances being between 2.70 and 2.87 Å. Thus, the overall arrangement is dominated by tight hemispherical clusters around the central sulphate ion. The water molecules serve mainly to act as a means of hydrogen-bonding the units together, with involvement of the proflavine amino-nitrogen atoms, as well as the charged sulphate ions. The overall hydrogen-bonding scheme has almost, though not quite, satisfied fully the hydrogen-bonding capability of the various groupings.

A distinctive feature of the crystal structure is the unimportance of ring system stacking. Of the four molecules in the asymmetric unit, only two overlap to any appreciable extent, although all four have their long axis pointing in roughly the same direction. There is no stacking between adjacent molecules in symmetry-related units. This situation contrasts notably that observed in the rather simpler proflavine dichloride crystal structure⁹, as well as in many other aminoacridines¹⁰.

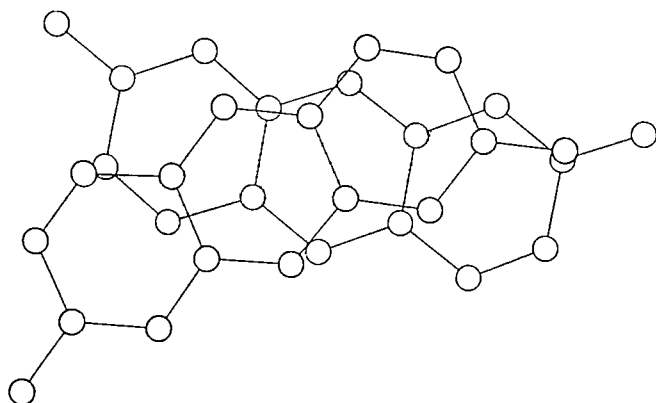


Fig 3 Skew stacking of two proflavine cations

These all have straightforward crystal structures with considerable parallel molecular overlap. The unusual skew stacking observed here is shown in Fig 3. The two ring systems are not related by any symmetry elements, crystallographic or non-crystallographic. One molecule is twisted relative to the other by 150°, and the charged nitrogens are on opposite sides. The two molecules are almost planar, with a separation of 3.34 Å.

It is interesting to note that proflavine dimerisation in solution has been suggested by a number of workers¹¹ to involve stacking. Whether this is similar to that observed here in the solid state, is of course uncertain. Our studies further suggest that close stacking and aggregation may be relatively unimportant in the external binding of proflavine to the phosphates of a DNA helix, certainly compared to any electrostatic interaction.

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Amino acid sequence similarity between cytochrome *f* from a blue-green bacterium and algal chloroplasts

"THE biological gap which separates bacteria and blue-green algae from all other cellular organisms represents one of the largest evolutionary discontinuities in the present day living world".¹ The origin of eukaryotic cells from simpler precursors² is an area of study where speculation is easier than experimental investigation. It seems unlikely that definitive fossil evidence for such early events will ever be obtainable, but molecular methods may be able to give some insight into evolutionary connections between eukaryotes and prokaryotes.

The blue-green algae, unlike other photosynthetic bacteria, carry out oxygenic photosynthesis. The whole photosynthetic apparatus of the blue-green algae bears much more resemblance to that of the chloroplast than it does to that of the other groups of photosynthetic bacteria. The chlorophylls, carotenoids and phycobiliproteins of the blue-green algae are particularly similar to those of the red algae.³ Similar electron transport components are found in both blue-green algae and in chloroplasts.⁴

Photosynthetic eukaryotes contain cytochrome *f* (ref. 5), which has a cytochrome *c*-type spectrum and a high redox potential and functions as one of the links between photosystems 1 and 2. In higher plants this cytochrome *f* is membrane bound and is isolated in an aggregated, although homogeneous form. The cytochrome of corresponding function can be readily isolated from many algae⁶ as a small acidic monomeric protein. Comparable cytochromes have been found in blue-green algae.^{7,8} Other types of photosynthetic bacteria contain a wide variety of cytochromes *c*, but none yet studied is very similar to the algal cytochromes *f* either in physical properties⁹ or in amino acid sequence.^{10,11} The cytochrome *c*-555 from green photosynthetic bacteria^{12,13} is the most similar in properties to the algal cytochromes.

Here we have isolated an *f*-type cytochrome from the blue-green alga, *Spirulina maxima*, and determined the amino sequence. We compare this sequence with the known sequences of algal cytochromes *f*, those of the chrysophyte *Monochrysis lutheri*¹⁴, the euglenoid *Euglena gracilis*¹¹, the phaeophyte *Alaria esculenta* (M. V. Laycock, personal communication), and the rhodophyte *Porphyra tenera* (R. P. A. and R. G. B., unpublished).

The cytochrome *f* was isolated from dried cells of *Spirulina maxima*. The cells were suspended in 0.06 M Tris-HCl, pH 8.0, and broken in a Manton-Gaulin homogeniser. The cytochrome was isolated from the 33-90% saturated ammonium sulphate fraction by chromatography on DEAE-cellulose at pH 8 and by gel filtration through Sephadex G-100. Phycobilins were eliminated with each operation. The cytochrome was finally separated into three fractions by chromatography on DEAE-cellulose (Whatman DE-11), adsorbing the protein from 0.001 M Tris-HCl, pH 8.0, washing the column with 0.01 M buffer, and finally separating and slowly eluting the cytochrome bands with 0.02 M buffer. Each cytochrome was then fractionated with ammonium sulphate and in each case the 60-80%

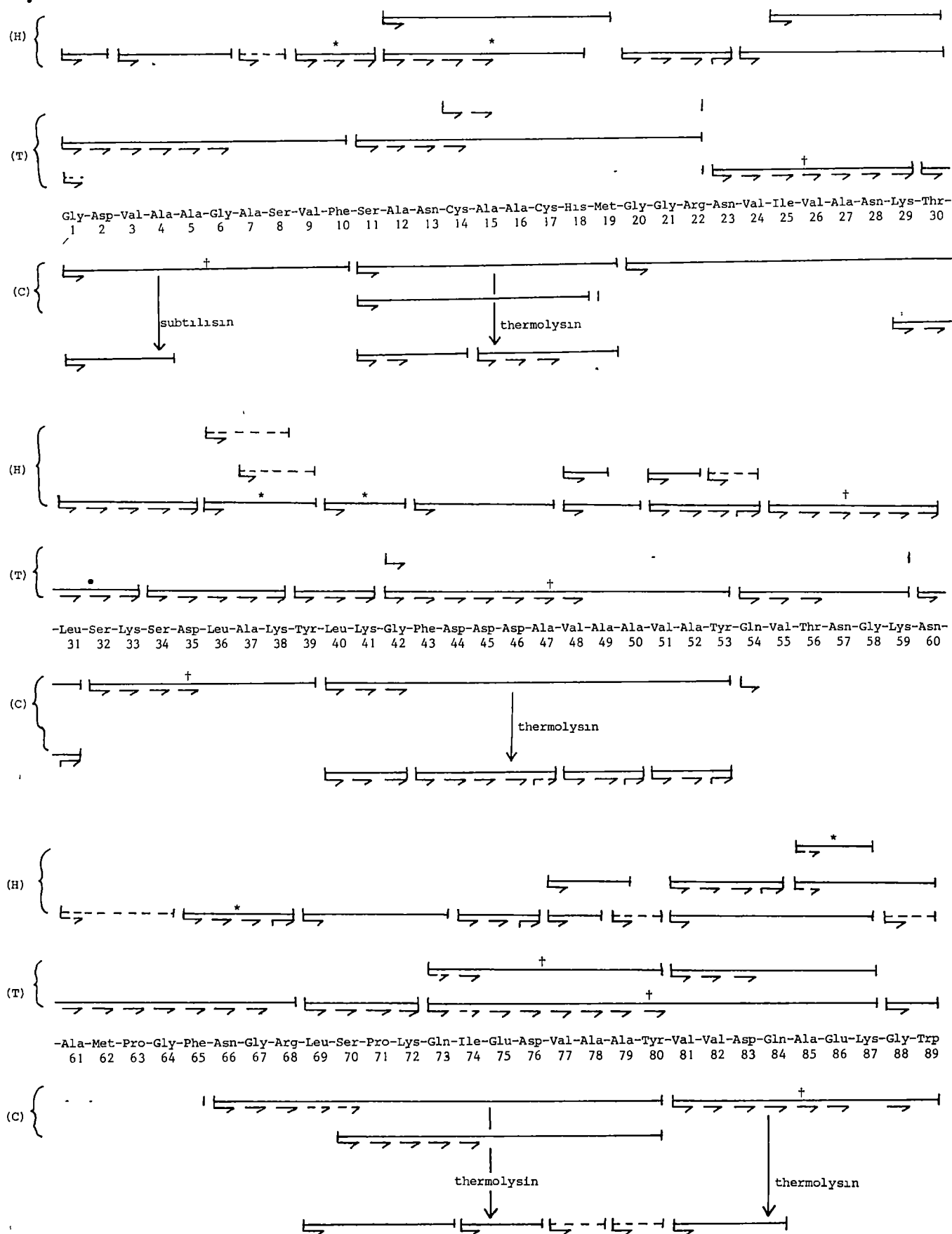


Fig. 1 Amino acid sequence of *Spirulina maxima* cytochrome *f*. Peptides derived by tryptic (T) and thermolysin (H) digestion are shown above the sequence, and by chymotryptic digestion (C) below the sequence. Vertical arrows show peptides produced by further digestion. —, Quantitative amino acid analyses (substandard^{15,16} if marked*), — — —, qualitative analyses, — — — — —, peptides that have definitely been identified as being present in digests but not isolated free from other peptides. — — — — — Indicates end groups and subsequent residues revealed by phenyl isothiocyanate degradation, and identified by the dansyl method (substandard^{15,16} if shown — — — — —), — — — — —, final residues of peptides identified as the free amino acid after the removal of the remainder by phenyl isothiocyanate degradation. † Peptides examined by carboxypeptidase A digestion.

saturated precipitate was collected. During purification, the buffers were kept 0.0001 M in mercaptoethanol or dithiothreitol, which kept the cytochromes in their reduced forms. The final yield of pure protein from 2.8 kg dry cells was about 18 μ mol cytochrome, divided among the three fractions. Portions of each of the three cytochrome fractions which were left standing for four months at 4°C in approximately 70% saturated ammonium sulphate containing 0.02 M Tris-HCl, pH 8.0, formed crystals.

The amino acid sequence was determined by methods similar to those used for some other prokaryotic c-type cytochromes^{15,16}. The haem was removed by treatment with HgCl₂ in 0.1 N HCl/8 M urea, the apoprotein digested with different proteases, and the peptides fractionated by gel filtration, paper electrophoresis and paper chromatography. Peptides were analysed quantitatively for amino acid composition and to assess purity, and sequences determined by the dansyl phenyl isothiocyanate method, by secondary digestion with other proteases and by investigation with carboxypeptidase A. Amide groups were assigned on the basis of electrophoretic mobilities of small peptides, and by the identification of the C-terminal amino acid of peptides by carboxypeptidase release or after the removal of all other amino acids by phenyl isothiocyanate degradation. The peptides isolated and the sequence deduced are shown in Fig. 1.

The proposed sequence contains two -Asn-Gly- sequences (57/58 and 66/67). Such sequences are well known to be particularly susceptible to deamidation¹⁷, especially at alkaline pH. The yields of peptides derived from these regions from the tryptic and chymotryptic digests were very low, and deamidated peptides were also recognised. For these digests, the apoprotein had been separated from urea and HgCl₂ by gel filtration in 0.1 M ammonia solution, as the apoprotein, in common with several other apocytochromes *f*, was insoluble in dilute acid and the peptides had also been eluted from paper with dilute ammonia. For the thermolysin digest, care was taken to avoid alkaline pH, the apoprotein was separated by gel filtration in 50% (v/v) formic acid, the enzymic digestion was carried out at pH 7.5, and peptides containing the susceptible sequences were eluted from paper with M acetic acid. This gave a good yield of the relevant peptides with electrophoretic mobilities that confirmed that asparagine and not aspartic acid was present. They could be quantitatively deamidated

by treatment with 2 M ammonia solution at 37°C for 5 h.

The three ionically distinct forms of the cytochrome *f* were separated by ion exchange chromatography in relative yields (in order of elution from DEAE-cellulose) of 30%, 60% and 10%, respectively. The forms were not distinguished spectrally or by amino acid analysis, and no differences were seen between the thermolysin peptide maps of the three apoproteins. It had been anticipated that the differences might be due to differential deamidation of the susceptible residues 57 and 66, but the peptide maps did not give any support to this hypothesis. The tryptic and chymotryptic peptides (Fig. 1) were isolated from the predominant form, from digests of 2.4 μ mol and 2.0 μ mol apoprotein respectively. The three forms were independently digested with thermolysin, but when the peptide maps showed no differences, the peptides were pooled for purification, resulting in 4.7 μ mol apoprotein for the combined digest. All the thermolysin peptides, even minor peptides isolated in very low yield, were compatible with the sequence shown in Fig. 1, and so there is at present no evidence as to the cause of the ionic differences between the three forms of the cytochrome *f*.

S. maxima cytochrome *f* has been under investigation in other laboratories. Before the present sequence studies began, a sequenator experiment (R. Holton and J. Ramshaw, personal communication) had identified residues 1-7, 9, 10, 12, 13, 15, 16, 23-25 and 27.

The known cytochrome *f* sequences are shown aligned in Fig. 2. It will be seen that there are several regions of good match, particularly around the haem attachment site (residues 14-17) and around methionine-62, the residue that is likely to function as the sixth iron ligand¹¹. In the middle of the sequences there are regions about twenty residues long (positions 34-52) which are very varied, and which are of slightly different lengths in different cytochromes *f*. Pettigrew¹¹ has discussed possible similarities between (*Euglena*) cytochrome *f* and other types of cytochrome *c* from bacteria and eukaryotes.

The sequences have not been compared by elaborate statistical methods, but a crude similarity matrix (Fig. 3) shows that the blue-green algal cytochrome *f* is at least as similar to each of the eukaryotic cytochromes *f* as the latter are to each other, and is considerably closer to the average sequence than is the *Euglena* protein. The distinctiveness of the *Euglena* sequence is in keeping with the many other

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
a	Gly	Asp	Val	Ala	Ala	Gly	Ala	Ser	Val	Phe	Ser	Ala	Asn	Cys	Ala	Ala	Cys	His	Met	Gly	Gly	Arg	Asn	Val	Ile	Val	Ala	Asn	Lys	Thr	Leu
b	Gly	Asp	Ile	Ala	Asn	Gly	Glu	Gln	Val	Phe	Thr	Gly	Asn	Cys	Ala	Ala	Cys	His	Ser	Val	Glx	Glx	Glx	Mml	Thr	Leu	Glu	Leu	Ser	Ser	Leu
c	Ala	Asp	Leu	Asp	Asn	Gly	Glu	Lys	Val	Phe	Ser	Ala	Asn	Cys	Ala	Ala	Cys	His	Ala	Gly	Gly	Asn	Asn	Ala	Ile	Met	Pro	Asp	Lys	Thr	Leu
d																															
e	Ile	Asp	Ile	Asp	Asn	Gly	Glu	Asp	Ile	Phe	Thr	Ala	Asp	Cys	Ser	Ala	Cys	His	Ala	Gly	Gly	Asn	Asn	Val	Ile	Met	Pro	Glu	Lys	Thr	Leu

	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62
a	-Ser	Lys	Ser	Asp	Leu	Ala	Lys	Tyr	Leu	Lys	Gly	Phe	Asp	Asp	Asp	Ala	Val	Ala	Ala	Val	Ala	Tyr	Gln	Val	Thr	Asn	Gly	Lys	Asn	Ala	Met
b	-Trp	Lys	-----	Ala	Lys	Ser	Tyr	Leu	Ala	Asn	Phe	Asn	Gly	Asp	Glu	Ser	Ala	Ile	Val	-----	Tyr	Gln	Val	Thr	Asn	Gly	Lys	Asn	Ala	Met	
c	-Lys	Lys	-----	Asp	Val	-----	Leu	Glu	Ala	Asn	Ser	Met	Asn	Thr	Ile	Asp	Ala	Ile	Thr	-----	Tyr	Gln	Val	Gln	Asn	Gly	Lys	Asn	Ala	Met	
d	-Ser	Lys	Thr	Ala	Ile	Glu	Glu	Tyr	Leu	Asp	Gly	Gly	Tyr	-----	Thr	Lys	Glu	Ala	Ile	Glu	-----	Tyr	Gln	Val	Arg	Asn	Gly	Lys	Gly	Pro	Met
e	-Lys	Lys	-----	Asp	Ala	-----	Leu	Ala	Asp	Asn	Lys	Met	Val	Ser	Val	Asn	Ala	Ile	Thr	-----	Tyr	Gln	Val	Thr	Asn	Gly	Lys	Asn	Ala	Met	

	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92
a	-Pro	Gly	Phe	Asn	Gly	Arg	Leu	Ser	Pro	Lys	Gln	Ile	Glu	Asp	Val	Ala	Ala	Tyr	Val	Val	Asp	Gln	Ala	Glu	Lys	Gly	Trp			
b	-Pro	Ala	Phe	Gly	Gly	Arg	Leu	Glu	Asp	Asp	Glu	Ile	Ala	Asx	Val	Ala	Ser	Tyr	Val	Leu	Ser	Lys	Ala	Gly						
c	-Pro	Ala	Phe	Gly	Gly	Arg	Leu	Val	Asp	Glu	Asp	Ile	Glu	Asp	Ala	Ala	Asn	Tyr	Val	Leu	Ser	Gln	Ser	Glu	Lys	Gly	Trp			
d	-Pro	Ala	Trp	Glu	Gly	Val	Leu	Ser	Glu	Asp	Glu	Ile	Val	Ala	Val	Thr	Asp	Tyr	Val	Tyr	Thr	Gln	Ala	Gly	Gly	Ala	Trp	Ala	Asn	Val
e	-Pro	Ala	Phe	Gly	Ser	Arg	Leu	Ala	Glu	Thr	Asp	Ile	Glu	Asp	Val	Ala	Asn	Phe	Val	Leu	Thr	Asx	Glx	Asx	Lys	Gly	Trp	Asp		

Fig. 2 Alignment of the amino acid sequences of cytochromes *f* from blue-green bacteria and eukaryotic algae. a, *Spirulina maxima* (blue-green bacterium), b, *Monochrysis lutheri*¹⁴ (chrysophycean alga), c, *Porphyra tenera* (red alga, R. P. A. and R. G. B., unpublished), d, *Euglena gracilis*¹¹, e, *Alaria esculenta* (brown alga, M. V. Laycock, personal communication). Mml, ϵ -N-methyl lysine. Amides at positions 5, 8 and 13 in sequence, b, have been assigned since original publication¹⁴ (M. V. Laycock, personal communication).

properties which divide the euglenoids from other algae¹⁸. The blue-green algal cytochrome *f* does not seem to be appreciably closer to any of the other bacterial cytochromes *c* than are the eukaryotic cytochromes *f*, but no rigorous searches for similarity have been made.

There seem to be three possible explanations for the close sequence similarity between the algal prokaryote and the algal eukaryote cytochromes *f*. First, the whole genome of the eukaryotic cells are evolutionarily derived from a prokaryote closely related to the blue-green alga, or, second, the eukaryotic chloroplasts are derived from a prokaryote related to the blue-green alga, but the remainder of the eukaryotic genomes are derived from different precursors, or, third, transfer of the cytochrome *f* genes (or of a cluster of genes) has taken place in one direction or other between the blue-green algal line and a eukaryote ancestor. The sequence similarity among the cytochromes *f* is so great that convergence cannot be considered a reasonable possibility.

On the present evidence it is not possible to distinguish between these three hypotheses, and it is not easy to design experiments that would rigorously distinguish between them. Much more sequence evidence will obviously be

	<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>	<i>e</i>
<i>a</i>	100	47	53	42	48
<i>b</i>	47	100	48	35	45
<i>c</i>	53	48	100	35	67
<i>d</i>	42	35	35	100	37
<i>e</i>	48	45	67	37	100

Fig. 3 Similarity matrix for amino acid sequences of cytochromes *f* from blue-green bacteria and eukaryotic algae. *a*, *Spirulina maxima*, *b*, *Monochrysis lutheri*, *c*, *Porphyra tenera*, *d*, *Euglena gracilis*, *e*, *Alaria esculenta*. The values shown are matches per 100 residues. For the comparison Asx is taken as being equal to both Asp and Asn, and Glx to both Glu and Gln.

needed, both from cytochromes *f* (particularly from other blue-green algae) and from other proteins and nucleic acids. It is important that parts of the blue-green algal genome concerned with functions other than photosynthesis should be compared with the corresponding parts in eukaryotes, for if the second hypothesis (the endosymbiont theory) were correct, the blue-green algal functions might be expected to have atrophied in the chloroplast. By some criteria, such as DNA base composition^{19,20}, the blue-green algae are a very heterogeneous group, but their photosynthetic mechanisms seem to be very much more uniform^{3,21}. This finding is consistent with the second and third hypotheses. Results from bacteria, particularly on the sporadic distributions of homologous proteins^{10,15,16}, suggest that much gene transfer has taken place. If such events (the third hypothesis) have affected a significant proportion of the genomes of a set of organisms over a period of time, then a phylogeny for the set as whole organisms will not exist. Sequence studies would then not be able to provide results from which a tree relating the major groups of organisms² could be deduced, but may be able to show that such trees are a meaningless oversimplification of what must actually have happened.

Very recently N-terminal sequence homology has been reported between the C-phycoerythrins of blue-green bacteria and the corresponding proteins of *Cyanidium caldarium*²², a eukaryotic alga of anomalous properties.

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M D Kamen. We thank Drs M D Kamen, T E Meyer and R W Holton for their interest and advice, and Dr M V Laycock for permission to include his unpublished *A. esculenta* sequence in Fig 2. *S. maxima* cells were a gift from Ing Hubert Durand-Chastel, Sosa Texcoco S A, Sullivan 51, Mexico 4, D F.

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Corrigendum

The Editor has been advised that the authorship of the paper "Synthesis mimics of insect juvenile hormone" (*Nature*, 232, 486, 1971) does not accurately reflect the work done on the paper. With the concurrence of all the present authors, the name of Hwalm Lee (then of Stauffer Chemical Company) should be added as first author.

Errata

In the Matters Arising contribution "Changes in the latitude of the climatic zones of the Northern Hemisphere" by M K Miles and C K Folland (*Nature*, 252, 616, 1974) the following corrections are necessary. The second author's name should read Folland not Follard, in the legend to Fig 2 1970–37 should read 1970–73 and in the legends to Figs 1 and 2 subpolar flow should read subpolar low.

In the article "Particle acceleration in planetary magnetospheres" by M J Houghton (*Nature*, 251, 205, 1974) equation (1) should read $\sigma_{\parallel} \approx \omega_{pe}/(4\pi \times 10^8)$ mho m⁻¹ and not as printed.

In the article "Acetylcholine as an excitatory neuromuscular transmitter in the stomatogastric system of the lobster" by E Marder (*Nature*, 251, 730, 1974) choline acetyltransferase was printed incorrectly as acetylcholine transferase on three occasions. These were in the heading to Table 1, in the first line of the penultimate paragraph on page 730 and in the second line of the last paragraph of the article.

reviews

Independence and Deterrence: Britain and Atomic Energy, 1945-1952. By Margaret Gowing. Vol. 1: *Policy Making*; pp. xi+503. Vol. 2: *Policy Execution*; pp. xiv+559. (Macmillan: London and Basingstoke, November 1974.) £10.00 each.

THESE two volumes of official history are notable for Professor Gowing's mastery of her subject, the orderliness of her presentation of a complex story and the clarity of her style. She has much to say which scientists will find absorbing, and for the non-scientist she manages to explain as much of the technology as he is likely to be able to assimilate. Even more valuably she reveals the intimate processes of government with a wealth of the sort of detail which the public is rarely allowed to know until at least 30 years have elapsed. The earlier release in this instance indicates how quickly this initial phase of atomic policy has passed into history. "Very little", we are told, "has been modified or omitted on public interest grounds".

Some early comments about the book, because they focused on Professor Gowing's more critical passages, gave the impression that her work was a revelation of governmental confusion and muddle, with parliament and the nation being kept in the dark while objectives were being pursued which had never been adequately discussed, even within the government or in Whitehall. The criticisms are there all right but the overall impression derived from reading the two volumes is different. The major decision which the new government took after 1945—to develop a national atomic programme, with a national atomic bomb as its top priority—did indeed emerge in a somewhat messy way. Most of the scientists and officials engaged in the preliminary arrangements seem to have acted on the assumption that they were intended

to produce atomic weapons, well before the ministerial decision was finally taken early in 1947. Parliament, meantime, had shown little curiosity and when it was formally told in May 1948 that atomic weapons were being developed there was scarcely a ripple on the political waters.

Once that decision had been taken, the necessary priority was given to the project and was subsequently upheld by Prime Minister Attlee whenever it needed to be re-asserted.

The result was the trial explosion of the bomb at Monte Bello in 1952, which was a major technical achievement. The only possible criticism of the execution of the policy—that it might have been completed sooner—is shown by Professor Gowing to be of little importance. Given the overall limitation of British resources in manpower, money and materials, any speeding up could have amounted to no more than

a few months. Indeed, in both timing and cost the operation came much closer to the original estimate made for it than most other projects involving frontier technology have been able to claim. It was undeniably a success story.

The only inside critics of any weight who questioned the decision to make the bomb, were Patrick Blackett and Sir Henry Tizard. Blackett's memorandum of 1947, reproduced in full in these books, was a clear statement of a particular point of view, and no doubt it deserved fuller discussion than it received. But its rejection is not surprising. For Blackett made assumptions about the likely attitudes of the United States and the Soviet Union and also about the likely effect upon other countries of Britain deciding not to make atomic weapons, which were shared by scarcely any politicians or civil servants at the time and by only

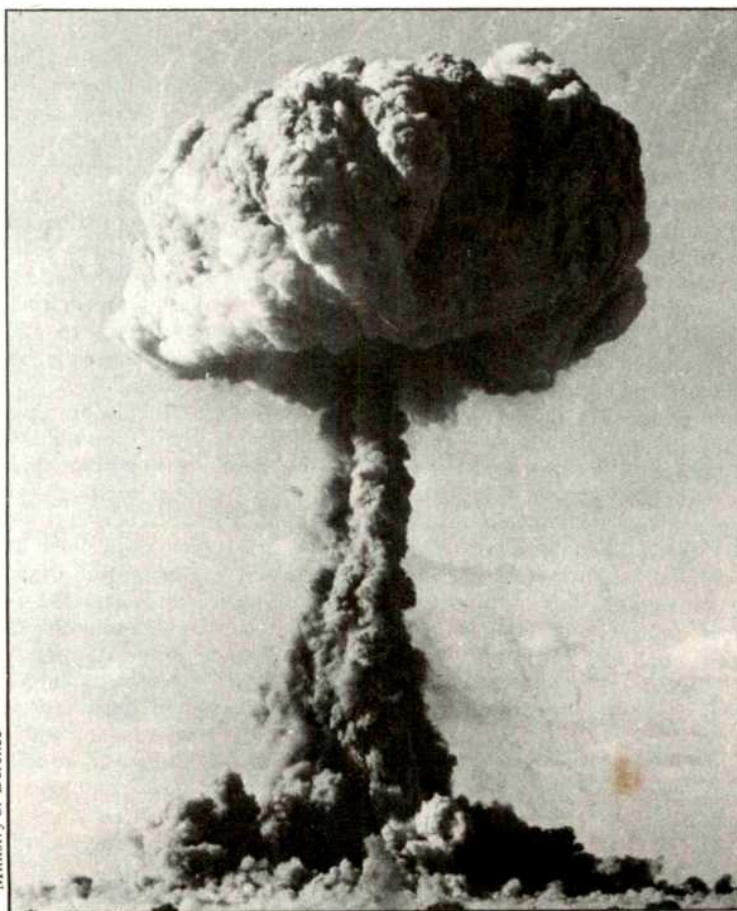
a few of his fellow scientists. Even in retrospect his forecasts of what might result if various alternative policies were followed seem little closer to what actually occurred than do the predictions of anyone else.

In 1949 Sir Henry Tizard, having perceived sooner than most that Britain's relegation from the ranks of the great powers was likely to be irreversible, argued that she could not afford this symbol of great power status, which she would in any case be unable to make effective. But it seems that he would have wished to divert the funds into other defence programmes rather than into civilian atomic projects, about which he was unduly pessimistic.

Despite Professor Gowing's admirably balanced narrative, it is not always easy for the reader to keep in mind the developing international scene which provided the background for atomic policy. At the beginning, the future attitudes of all the wartime allies were in doubt; not the least of

The way it happened

Kenneth Younger



Ministry of Defence

these doubts concerned the likely attitudes of the US and USSR to one another. By the end of 1947 the Council of Foreign Ministers had finally broken down and anxiety about Soviet Policy in Europe was growing, reaching a climax with the Berlin blockade in the following year. British politicians, who had only just emerged from the second world war, found the outbreak of a third entirely credible. Moreover, they remembered just how it had felt in the pre-war years to be militarily outclassed by a potential enemy; and the only way to avoid a repetition of that was to cling to the American alliance. They were, however, still unsure, even in 1949, of permanent American military commitment in Europe and therefore found it impossible to contemplate throwing away the one major weapon in the development of which, they had reason to believe (though wrongly as it turned out), they were ahead of the USSR.

As regards the implementation of the programme, the heroes of the story are unquestionably the three men who led the main institutions entrusted with research, production of fissile material and weapons: John Cockcroft, Christopher Hinton and William Penney. All were supremely competent in their respective specialities and all showed an exceptional capacity for leadership of their teams. What stands out from Professor Gowing's story is that the quality of these men and their capacity to cooperate with one another triumphed over the complicated bureaucratic framework within which they had to operate. Even the absence, at certain periods, of clear central direction and coordination may have had its compensations in the flexibility which it allowed the scientists in changing their methods according to the state of the art. The supervising department—the Ministry of Supply—also deserves credit for having been capable of adjusting many civil service practices to the needs of a wholly unprecedented pioneering operation. In contrast, industry, with a few honourable exceptions, proved unimaginative and inadequate.

At the point when these volumes break off in 1952 a new phase, on which we are promised further volumes, was opening. Slowly the implications of the new weaponry—moral, political and military—came under public discussion. Almost equally slowly a realisation of Britain's changed world status came home to public opinion and with it an altered appreciation of the comparative importance of Britain's relations with the United States (which dominates the volumes already published), the Commonwealth and Europe.

How far the policies followed from

1945–52 may have prejudiced the subsequent evolution of British atomic policy, for better or for worse, will no doubt emerge as the story unfolds. 'Very little' would be my present verdict. What was done, I believe, in the immediate post-war years had to be done in the confused circumstances of that time, and in that sense was correct. The next phase was entirely different. Events such as the end of the Stalin era, the coming of the hydrogen weapon and the space programmes, and the rapid reduction of Britain's responsibilities as a world power from the late 1950s onwards combined to require a reassessment of Britain's role in nuclear weaponry.

Whether the choices which Britain then made were wise or not, we shall

hope to learn from later volumes, but I do not think that they were prejudged by what had gone before, except in the sense that if Britain had had no atomic programme she would have had little or no choice to make. For myself, having spent the years 1945–52 as a Labour MP, with a spell in the Foreign Office as a Minister (1950–51), I am a little surprised to find nothing in these volumes which, had I known of it at the time, would have radically altered my view of the role Britain should play. Nor would I have dissented from the atomic programme which was under way.

If this sounds a little like saying that because it happened this way, this was the way it had to happen, I can't help that. □

Varying aspects of fisheries work

Sea Fisheries Research. Edited by F. R. Harden Jones. Pp. xvii+510. (Elek Scientific Books: London, March 1974.) £10.00.

THIS book is a collection of 21 essays in honour of Michael Graham, former Director of Fishery Research with the Ministry of Agriculture, Fisheries and Food at Lowestoft. Graham commenced his career in fisheries research in 1920, and his early work on the North Sea cod and its fishery was, and still is, a model of investigation into fish biology, life cycles, spawning grounds, and age compositions. The study led to proposals for the better management of the fishery and was an outstanding contribution to the so-called theory of fishing, whereby attempts are made to establish parameters for growth, recruitment, and natural and fishery mortality. After the 1939–45 war Graham returned to Lowestoft as Director of Fishery Research, and immediately began planning for the postwar research effort by building vessels and recruiting staff of a calibre which has made the Fisheries Laboratory at Lowestoft, and its satellites at Conwy and Burnham-on-Crouch, respected throughout the world. Graham retired in 1958 and characteristically applied himself to the environmental reclamation of colliery waste tips, and to lecturing. Sadly, he did not live to see the publication of this book, but one feels that he would have approved of this collection of essays by his former colleagues.

Naturally, the varying aspects of fisheries work are well covered. Papers on the exploitation of stocks of fish and shellfish are much in evidence. M. J. Holden's thoughtful paper on the rational exploitation of elasmobranch populations suggests how vulnerable to extinction most of them would become

if heavily exploited. That is supported by A. C. Burd's requiem for the North East Atlantic herring and its virtually extinct fishery, a classical example of the failure of some fishery scientists to accept the evidence of others, and the consequent lack of international co-operation. The world resources of hakes of the genus *Merluccius* are discussed by B. W. Jones, who suggests that several species are still underexploited, although some, notably the European hake (*M. merluccius*), have been heavily overfished. In an essay on the world's industrial fisheries (that is, fisheries which produce fish meal and oil), C. T. Macer confirms that some hakes, especially the western South American species-group (*M. gayi*), might support a greater fishery than at present. A contribution from P. R. Walne discusses the problems of the shellfish industries of England and Wales. He also considers the fascinating possibilities allowed by the development of successful culture techniques, for which most of the biological requirements have already been established.

It is suspected that the pollution of inshore waters is one of the factors responsible for the low rate of recruitment of young individuals to many native shellfish populations. In the Essex oyster fishery the total catch declined as the human population, and the consequent pollution of the area, increased. H. A. Cole takes a broader look at pollution in his essay on marine pollution and the UK fisheries, and he concludes that there is little evidence that the discharge of industrial and domestic waste into the sea has had a deleterious effect on sea fisheries. In support of this he cites the increase in landings of plaice, cod, and haddock in the North Sea, and plaice and cod in the Irish Sea (both are enclosed and relatively polluted seas). Changes of

this nature may be, however, a direct result of fluctuations in year-class strength, and of changing climatic conditions, both factors may mask the adverse effects of pollution

Several of the essays are concerned with technical subjects R W Blacker's discussion of recent advances in otolith studies is an important contribution to the methods of study and interpretation of otoliths for age determination (a 'traditional' fisheries' tool) By contrast, Alan Jamieson and C E Purdom present new techniques in fisheries research with essays on the study of genetic tags for marine fish stocks, and genetic variation in fish The volume also contains a contribution on the design of research vessels by Geoffrey C Trout, as important a study as any attempted at Lowestoft (and as one who has sailed on FRV *Cirolana*—the newest of the fleet of research vessels—I can vouch for the excellence of the product

The quality of production of this book is excellent, although the photographic illustrations do not reproduce well The editing is even and unobtrusive and must have represented a formidable task for the editor Altogether this is an admirable and fitting volume in honour of Michael Graham

Alwyne Wheeler

Liquid crystals

The Physics of Liquid Crystals By P G de Gennes (The International Series of Monographs on Physics) Pp xi+333 (Clarendon Oxford, Oxford University Press London, June 1974) £11.50

THIS is the first modern book to survey and discuss the physical properties of liquid crystals Liquid crystals combine some of the properties of the crystalline and of the isotropic liquid phases, they have received considerable attention during the last 10 years The re-discovery of electro-optical effects, which are used in liquid crystal display devices, has led to a revival of basic research on the physical and chemical properties of liquid crystals, both theoretically and experimentally Their study is complicated because it involves various physical and chemical disciplines, making it almost impossible to cover all the aspects in one book Although the main physical topics are discussed or at least mentioned in this book, the main emphasis is on hydrostatic and hydrodynamic properties which, in principle, also determine the optical properties

In Chapter 1 there is a short introduction to the nature of the different liquid crystalline phases and of the constituent molecules The following four

chapters, the major part of the book, deal with the nematic liquid crystalline phase which is characterised by long range, orientational ordering of the constituent, rod-like molecules

Chapter 2 deals with long and short range order Different order parameters are introduced and linked to measurable quantities, such as nuclear magnetic resonance spectra, the anisotropy of magnetic susceptibility, and so on Some theories of orientational ordering are reviewed briefly Finally, short range order effects near the transition from the isotropic to the nematic phase are discussed The static, elastic distortions in a nematic single liquid crystal are treated in Chapter 3 There, a hydrostatic theory based on a continuum description is introduced and discussed thoroughly, and the role of boundary effects and boundary conditions is made clear Various applications of the theory, and some instructive problems are given Most are concerned with deformations induced by external magnetic and electric fields for different boundary conditions Close attention is also given to the strong natural light scattering, which is analysed in terms of spontaneous orientation fluctuations

Chapter 4 describes distortions that are not continuous but which involve singular points or lines These defects, where the orientation varies discontinuously, are called disclinations In connection with disclinations different textures are discussed

An understanding of the coupling between orientation and flow is essential to any consideration of hydrodynamic properties That problem, and the different approaches to it, are discussed thoroughly in Chapter 5 Again various applications and experimental situations are described, including classical flow experiments, flow induced by external fields, inelastic light scattering, dynamic scattering, and so on

The cholesteric liquid crystalline phase, essentially a helically distorted nematic phase, is also discussed Because of the helical structure this phase has rather peculiar optical properties which are discussed in a rather formal way The hydrostatic and hydrodynamic theories of the nematic phase are extended to a discussion of the cholesteric phase The static distortion of the helical structure by external fields, flow properties, the analogue of dynamical scattering and other typical phenomena are considered and some typical defects and textures are also described

The last chapter is devoted to smectic liquid crystals In addition to the orientational ordering they have a layer structure First, a classification and description of the different smectic phases is given, and then attention is restricted

to the A and C-phases In the discussion of the static and dynamic properties the role of the layer structure is clearly displayed Again, attention is given to distortions induced by external fields and mechanical forces, to light scattering, the propagation of acoustic waves and flow properties The chapter ends with a description of phase transitions and precritical phenomena

Though various experimental methods and experiments are mentioned or briefly described, the main emphasis is on the theoretical aspects The approach to these aspects, which is purely phenomenological, is based on the fundamental hydrostatic and hydrodynamic equations The study of chapters 3 and 5, which form the central part of the book, is therefore necessary before the chapters on the cholesteric and smectic phases can be understood One may regret that little or no attention has been given to molecular theories and considerations, as these have been quite successful and instructive Some of the discussions are rather formal and general, whereas others are more concise, but the material is nevertheless presented in a clear and systematic way

This is a most valuable book which gives a comprehensive though not easy introduction to many aspects of the liquid crystalline phases

W. J. A. Goossens

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Edited by R H STÖY
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Energy and fat

Energy Balance and Obesity in Man
By J S Garrow Pp xii+335 (North
Holland Amsterdam, London, American Elsevier New York, 1974) Dfl.80,
\$30.80

THIS is a very well written, highly persuasive, important book which provides all the basic and practical information necessary for an understanding of the problem of obesity.

The trouble with 'obesity' is that it produces emotional reactions which people rationalise from a literature that provides any evidence desired. In medical science there can be few pursuits which affect such large numbers of people with such little effect, as the investigation and treatment of obesity. Of course, few things are as satisfying to the intellectual academic as a problem with little prospect of application to a real-life situation, and that may explain the large volume of contradictory literature on this subject.

In a slightly lengthy introduction, Dr Garrow initiates us into his positive interpretation of this mass of confusing information. His book reviews comprehensively the different methods used to measure the intake and expenditure of energy in man, the theories of the control of energy intake, the part played by physical activity, energy expenditure, and the variable composition of the human body in relation to fat and how this can be measured. The final section is concerned with the difficult, practical problems of how to diagnose and classify an obese patient and includes a critique of the likely effectiveness of the various forms of treatment.

My only serious criticism of this excellent book is that, possibly because the author has apparently become actively interested in obesity only comparatively recently, his interpretation frequently seems biased and somewhat selective. He fails to quote certain relevant literature, especially from the large volume of physiological papers on exercise, and he is perhaps slightly too dogmatic in many of his statements. In those sections in which he has first-hand knowledge—for example, the chapter on "Energy stores: their composition, measurement and control"—he writes clearly and dispassionately in a most stimulating fashion.

I have no hesitation in recommending this book. To my knowledge, it is by far the best book on the subject of energy balance in man. It should be read by everyone involved in the study of obesity—though all should retain certain reserves about accepting totally some of Dr Garrow's views.

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obituary

S. Petterssen

SVERRE PETTERSSSEN, the outstanding meteorologist, died in London on December 31. He was 76.

Born in Norway, Petterssen served in the Norwegian Meteorological Service from 1924 to 1939 after graduating from Oslo University. In 1939 he became Professor of Meteorology at the Massachusetts Institute of Technology, and came to England in 1942. During the war he was stationed at the Meteorological Office at Dunstable, establishing now standard techniques of upper air analysis. In recognition for his services, he was made CBE in 1948 and Commander of the Order of St Olaf in 1949.

After the war Petterssen returned to Norway, serving in the Norwegian Weather Forecasting Service until 1948, when he was appointed Director of Scientific Services of the US Air Force Weather Service. In 1952, he returned

to academic life as Professor of Meteorology at the University of Chicago. In the next ten years, he played a large part in the rapid expansion of research and education within government and academic institutions.

Petterssen's contributions to the theory of frontogenesis and convection, to the kinematics of weather systems and to the physics of fog were highly original, and led the way towards more quantitative methods of weather forecasting.

H. Heller

HANS HELLER, the distinguished endocrinologist, died on December 30 at the age of 70.

Born in Brno, Professor Heller studied Natural Sciences at Cambridge from 1929 to 1931, when he was

appointed to a post in pharmacology at the University of Vienna. He returned to England in 1935 to obtain medical qualifications at University College Hospital. He worked there as a Beit Fellow until 1941, when he took an appointment at Bristol University, becoming Professor of Pharmacology in 1949.

Heller was particularly famous for his investigations of the water balance effect of neurohypophyseal hormones in amphibia, showing that the water-balance principle differed from that of mammals, later isolating the hormones from cod in 1959. His research into the nature of such hormones in other animals illustrated a pattern for the evolutionary development of the hormones.

From 1963 to 1974, he was editor of the *Journal of Endocrinology* and at the time of his death was chairman of the Society for Endocrinology.

announcements

Awards

New Year's Honours

Max Ferdinand Perutz has been made a Companion of Honour for services to molecular biology.

Knights Bachelor include **Arthur Llewellyn Armitage**, vice-chancellor, Victoria University of Manchester, **Frederick Arthur Bishop**, Director-General, National Trust, **Harold Montague Finnieston**, FRS, chairman, British Steel Corporation, **Hugh Ford**, FRS, Professor of Mechanical Engineering, Imperial College of Science and Technology, **Ieuan Maddock**, FRS, chief scientist, Department of Industry, **Edward Eric Pochin**, lately director, MRC Department of Clinical Research.

Erik Erikson, Professor of Psychiatry at Harvard University, has been awarded the **Mental Health Association Research Achievement Award** at the organisation's annual meeting in Washington.

Godfrey Hounsfield has been awarded the **Wilhelm Exner Medal**, a major Austrian award, for the invention of the EMI computerised X-ray brain scanner.

Appointments

Henry Francis Black has been appointed to a personal chair of mechanical engineering at Heriot-Watt University.

Miscellaneous

Mitchell Prize The Mitchell Energy & Development Corporation, in association with the Club of Rome, is to make biennial awards for papers submitted on the problems inherent in the transition from growth to equilibrium of population, material consumption and energy use over the next 40 years. Application deadline for 1975 is February 28. For information contact **Limits to Growth '75**, 5645 South Woodlawn Avenue, Chicago, Illinois 60637.

The Food and Drug Administration's OTC Panel is interested in receiving any unpublished data on foetal damage or loss associated with the maternal use of vaginal contraceptives or douches. For information, contact **Elizabeth B. Connell**, Food and Drug Administration Bureau of Drugs, Division of OTC Drug Evaluation (HFD-510), 5600

Fishers Lane, Rockville, Maryland 20852.

The **Moscows Seminar on Collective Phenomena** will be held every Sunday at noon, on a broad range of scientific topics. For information, contact **I and V. Brailovsky**, pr Vernadskogo 99, korp(bldg) No 1, kv (Apt) 128, Moscow.

Lady Tata Memorial Trust Applications are invited for scholarships and fellowships to aid research on leukaemia and allied conditions. Further information may be obtained from Secretary (European) Scientific Advisory Committee, Lady Tata Memorial Trust, Chester Beatty Research Institute, Fulham Road, London SW3.

A. E. Bennett Research Awards The Society of Biological Psychiatry is offering two awards—one in basic and one in clinical science—for the purpose of stimulating worldwide research in biological psychiatry by young (under 35 years) investigators. Further information from Chairman, Committee on Research Awards, Veterans' Administration Hospital, Brockton, Massachusetts 02401.

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International meetings

March 13-14, **Chromosomal Proteins and Their Role in Regulation of Gene Expression**, Florida (Florida Colloquium on Molecular Biology, Department of Biochemistry, University of Florida, Gainesville, Florida 32610)

April 3-6, **Electrochemistry Symposium**, London (Hon Secretary, Felix Gutmann, Department of Physical Chemistry, The University of Sydney, NSW 2006, Australia or Dr D Inman, Imperial College, London, UK)

April 7, **Metal Compounds in Biological Systems**, Sheffield (Dr E D McKenzie, Department of Chemistry, University of Sheffield or Dr G J Leigh, A R C Unit of Nitrogen Fixation, University of Sussex, UK)

April 7-9, **Food from Waste**, Weybridge (The Secretary, National College of Food Technology, Weybridge, Surrey, UK)

April 7-9, **Interatomic Forces in Condensed Matter**, Reading (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1X 8QX, UK)

April 7-14, **Recycling and Disposal of Solid Waste**, Dubrovnik (Mr R V Arnfield, Director, Industrial and Business Liaison Office, University of Nottingham, University Park, Nottingham, NG7 2RD, UK)

April 8-10, **Thermal Regime of Glaciers and Ice Sheets**, Vancouver (R B Sagar, Department of Geography, Simon Fraser University, Burnaby, B C V5A 1S6, Canada)

April 8-11, **Environmental Biochemistry**, Burlington (Dr Jerome O Nriagu, Department of the Environment, Canada Centre for Inland Waters, Burlington, Ontario L7R 4A6, Canada)

April 8-11, **Organ Cultures in Biomedical Research**, Norwich (Dr B M Richards, Searle Research Laboratories, Lane End Road, High Wycombe, Bucks, HP12 4HL, UK)

April 14-17, **Nuclear Quadrupole Resonance Spectroscopy**, Tampa, Florida (3rd International Symposium on Nuclear Quadrupole resonance, Department of Physics, University of Florida, Gainesville, Florida 32611)

Index of Current Government and Government-Supported Research in Environmental Pollution in Great Britain, 1973 Pp 206 (London Department of the Environment, 2 Marsh Street, SW1P 3EB, 1974) [1111]

The Universities and Applied Research Their Relevance to Social and Industrial Needs (Proceedings of the 1974 Symposium held on 23rd April at the Royal Society, London) Pp 90 (London The Research and Development Society, 47 Belgrave Square, SW1X 8QX, 1974) £2.50 members, £4.50 non-members [1111]

Report from the Select Committee on Science and Technology—Offshore Technology (Session 1974) Pp 53 (London HMSO, 1974) 51p net [1211]

Dinosaurs By W E Swinton Fifth edition Pp vii + 47 (9 plates) (London British Museum (Natural History), 1974) 50p [1211]

Another Sword for St James By Professor Derek W Lomax (Inaugural Lecture delivered in the University of Birmingham on 19th February 1974) Pp 19 (Birmingham The University, 1974) 25p [1211]

Unconventional Protein Sources A Guide to Selected Literature and Sources of Information Pp 8 Automobile Fuels—The Alternative to Petroleum A Guide to Selected Literature Pp 10 Salmon and Trout A Guide to Selected Literature and Sources of Information Pp 11 (Guidelines) (London Publications, Science Reference Library (Bayswater Branch), 10 Porchester Gardens, 1974) gratis [1311]

Computer Board for Universities and Research Councils Report of the Computer Board for the period 1st April 1972-31st March 1974 (Cmnd 5775) Pp 25 (London HMSO, 1974) 26p net [1411]

Institute for Marine Environmental Research Report 1973/1974 Pp 71 (Plymouth Institute for Marine Environmental Research, 1974) [1411]

Philosophical Transactions of the Royal Society of London A Mathematical and Physical Sciences Vol 277, No 1268 Topography and Topology in Solid-State Chemistry By J M Thomas Pp 251-286 + plates 6-11 (London The Royal Society, 1974) [1411]

Department of the Environment Annual List of Publications 1973 Pp vi + 121 (London Department of the Environment, 1974) [1511]

Royal Observatory Annals, No 8 Photoheliographic Results 1965 Pp 42 (Herstmonceux Royal Greenwich Observatory, 1973) 90p net [1511]

1974/1975 ICI Information Handbook Pp 48 (London ICI, Ltd 1974) [1811]

Pharmaceutical Industry Press Directory Pp 23 (London The Association of the British Pharmaceutical Industry, 1974) [1811]

The Scientific Proceedings of the Royal Dublin Society Series A Vol 5, No 11 Poaceae—Irish Members Part 5 Anatomy By M A Farragher Pp 159-198 + plates 17-28 £1.50 Vol 5, No 12 Geological Evidence for the Original Nature of the Ross-Lake Complex, S E Eire By R S Thorpe Pp 199-206 40p Vol 5, No 13 The Glaciations of the Dingle Peninsula, County Kerry By Colin A Lewis Pp 207-236 £1 Vol 5, No 14 The Evolution of North Bull Island, Dublin Bay By Colin R Harris Pp 237-252 + plates 29 and 30 50p Vol 5, No 15 Glacial Retreat of Late Midlandian Ice in the Slieve Bernagh By T F Finch Pp 253-264 60p Series B Vol 3, No 19 A Laboratory Culture Medium for *Rhizobium* from *Lotus pedunculatus* By M B Walsh and P L Curran Pp 267-272 + plate 12 20p (Dublin Royal Dublin Society, 1974) [1811]

The Zoological Record 1971, Vol 108, Section 18 Aves Compiled by the Staff of the Zoological Society of London Pp 264 £11 1971 Vol 108 Section 19 Mammalia Compiled by the Staff of the Zoological Society of London Pp 405 £12.50 (London The Zoological Society of London 1974) [1811]

University of Nottingham Report of the School of Agriculture, 1973/1974 Pp 170 (Sutton Bonington Loughborough University of Nottingham, School of Agriculture 1974) £1 [2011]

The National Institute of Agricultural Botany Report and Accounts, 1973 Pp 89 (Cambridge The National Institute of Agricultural Botany 1974) [2011]

The Radiochemical Centre Technical Bulletin 74/3 Mossbauer Sources Pp 20 (Amersham The Radiochemical Centre 1974) [2111]

Philosophical Transactions of the Royal Society of London B Biological Sciences Vol 269, No 898 Evolution and Adaptive Radiation in the Mactrochidae (Crustacea Cladocera)—A Study in Comparative Functional Morphology and Ecology By G Fryer Pp 137-274 (London The Royal Society 1974) [2111]

National Institute of Agricultural Engineering Annual Report, 1st April 1973-31st March 1974 Pp 80 (Silsoe Bedford and Penuick, Midlothian National Institute of Agricultural Engineering 1974) £2 [2211]

Greater London Council Scientific Branch Annual Report of the Scientific Adviser 1973 By R T Kelly Pp 140 (London Greater London Council, 1974) £4.25 [2211]

Elements of Organometallic Chemistry By F R Hartley (Chemical Society Monographs for Teachers, No 26) Pp vi + 103 (London The Chemical Society 1974) £1.20 [2511]

Other countries

National Research Council of Canada Report of the President, 1973/1974 Pp 133 (Ottawa National Research Council of Canada 1974) [4111]

CERN—European Organization for Nuclear Research CERN 74-19 Supermix—a Multi-Host Front End Concentrator System for Asynchronous Consoles By T Bruins K S Olofsson E M Palindri B Segal, H J Slettenhaar and H Strack-Zimmermann Pp vii + 85 (Geneva CERN, 1974) [4111]

Neurophysiology of Enlightenment Scientific Research on Transcendental Meditation Presented by Dr Robert Keith Wallace Pp 48 (Seelsberg, Switzerland Maharishi International University, 1974) [4111]

Canada Department of Energy, Mines and Resources Geological Survey of Canada Memoir 378 Geology of Bathurst Island Group and Byam Martin Island, Arctic Canada, (Operation Bathurst Island) By J Wm Kerr Pp 152 (18 plates) \$5 Paper 73-38 Research in Geochemical Prospecting Methods for Fluorite Deposits, Madoc Area, Ontario By J P Lalonde Pp 56 \$3 Paper 73-40 Late-Wisconsin Glaciation of Southwestern Newfoundland (with Special Reference to the Stephenville Map Area) By I A Brookes Pp 31 \$3 (Ottawa Information Canada, 1974) [4111]

Problems and Programmes Related to Alcohol and Drug Dependence in 33 Countries By Joy Moser Pp iii + 90 (Geneva World Health Organization, London HMSO, 1974) Sw fr 20 [4111]

Smithsonian Contributions to Zoology No 166 A Checklist of the North and Middle American Crayfishes (Decapoda Astacidae and Cambaridae) By Horton H Hobbs Jr Pp iii + 161 \$2.50 No 175 On the Caobangidae, a New Family of the Polychaeta, with a Redescription of *Caobangia billeti* Girard By Meredith L Jones Pp iii + 55 \$1.15 (Washington, DC Smithsonian Institution Press, 1974 For sale by US Government Printing Office) [7111]

United States Department of the Interior Geological Survey Water-Supply Paper 1850-D Floods of June 1965 in Arkansas River Basin, Colorado, Kansas, and New Mexico By R J Snipes, et al Pp iv + 97 + 2 plates \$2.35 Water-Supply Paper 2031 Influence of Recharge Basins on the Hydrology of Nassau and Suffolk Counties, Long Island, New York By G E Seaburn and D A Aronson Pp vi + 66 + 1 plate \$1.75 (Washington, DC Government Printing Office, 1974) [8111]

World Health Organization—Regional Office for Europe Copenhagen Report of the Regional Director, July 1973 to June 1974 Pp 129 (Copenhagen WHO, Regional Office for Europe, 1974) [8111]

Giornale Italiano di Psicologia (Italian Journal of Psychology), Vol 1, No 1, Aprile 1974 Pp 1-114 Published 3 times yearly Subscription Lit 6500 Italy, Lit 8000 abroad Single issues Lit 2500 (Bologna Societa Editrice il Mulino, Via Santo Stefano 6, 1974) [1111]

Norsk Polarinstittut Skriften Nr 158 Contribution to the Geology of North Wester Spitsbergen By A Hjelte and Y Ohta Pp 107 + 10 plates Nr 189 Palaeogene Deposits and the Platform Structure of Svalbard By Ju Ja Lissic Pp 50 Nr 160 The Ordovician Trilobites of Spitsbergen By I Olenidae Pp 80 + 24 plates (Oslo Norsk Polarinstittut, 1974) [1111]

Effects of Agricultural Production on Nitrates in Food and Water with particular reference to Isotope Studies (Proceedings and Report of a Panel of Experts organised by the Joint FAO/IAEA Division of Atomic Energy in Food and Agriculture and held in Vienna, 4-8 June 1973 Panel Proceedings Series) Pp 158 (Vienna IAEA, London HMSO, 1974) 146 Austrian schillings, £3.40, \$8 [1211]

World Population Projections Alternative Paths to Zero Growth (*Population Bulletin*, V 1 29, No 5) Pp 32 (Washington, DC Population Reference Bureau, Inc., 1974) 50 cents [1211]

William Roxburgh's Fern Types By C V Morton (Contributions from the United States National Herbarium, Vol 38, Part 7) Pp 283-396 (Washington, DC Smithsonian Institution Press 1974 For sale by US Government Printing Office) \$1.70 [1211]

Canada Department of Energy, Mines and Resources Geological Survey of Canada Paper 74-3 1973-1974 Index of Publications of the Geological Survey of Canada Pp 47 \$2 Paper 74-4 Abstracts of Publications in Scientific Journals by Officers of the Geological Survey of Canada, April 1973-March 1974 Pp 30 \$2 Paper 74-8 Geology and Mineral Deposits of Alerte-Cape Scott Map-Area, Vancouver Island, British Columbia By J E Muller K E Northcote and D Carlisle Pp 77 \$4 Paper 74-10 Contribution to the Jurassic and Cretaceous Geology of Northern Yukon Territory and District of Mackenzie Northwest Territories By J A Jeletzky Pp 23 \$3 Paper 74-23 Paleontology of Two Sections of Late Quaternary Sediments from the Porcupine River, Yukon Territory By Sigard Licht-Federovich Pp 6 \$2 Paper 74-44 Project Egma Seismic Survey—Timmins Ontario to Val d'Or, Quebec By P G Killen and G D Hobson Pp 33 \$2 Paper 74-50 Stratigraphy of the Shell Naskapi N-30 Well, Scotian Shelf, Eastern Canada By G L Williams, L F Jansa D F Clark and P Ascoli Pp 12 \$2.50 (Ottawa Information Canada, 1974) [1211]

National Research Council Canada NRCC No 13690 The Application of the Phosphorus Loading Concept to Eutrophication Research By R A Vollenweider and P J Dillon Pp 42 (Associate Committee on Scientific Criteria for Environmental Quality) (Ottawa Publications, NRC 1974) \$1.25 [1211]

Fisheries Research Board of Canada Technical Report No 482 A Bibliography of Canadian Atlantic Coast Estuary Studies By R O Brinkhurst, L Linkletter S Connors and A Hamilton Pp 23 (St Andrews NB Research and Development Directorate, Biological Station 1974) [1211]

Isotope Studies on Wheat Fertilization (Results of a Four-Year Co-ordinated Research Programme of the Joint FAO/IAEA Division of Atomic Energy in Food and Agriculture Technical Reports Series, No 157) Pp 100 (Vienna IAEA London HMSO 1974) 92 Austrian schillings, £2.10 \$5 [1211]

World Meteorological Organization Technical Note No 132 Applications of Meteorology to Economic and Social Development By R Schneider J D McQuigg L L Means and N K Kiyukin Pp xiv + 130 (Geneva World Meteorological Organization, 1974) [1311]

Reports and Publications

Great Britain

Philosophical Transactions of the Royal Society of London B Biological Sciences Vol 269, No 897 Implantation, Foetal Membranes and Early Placentation of the African Elephant *Loxodonta africana* By J S Perry Pp 109-136 + 1-7 (London The Royal Society, 1974) [8111]

British Nutrition Foundation Annual Report 1973/1974 Pp 26 (London British Nutrition Foundation 93 Albert Embankment, SE1, 1974) [1111]

Rearing the Hymenoptera Parasitica By K G V Smith Pp 15 (AES Leaflet No 35) (London Amateur Entomologists' Society, 137 Gleneldon Road, SW16, 1974) 40p [1111]

nature

January 31, 1975

Amber light for genetic manipulation

THE report of Lord Ashby's working party (Cmnd 5880, HMSO, 26p) makes it seem more likely than not that biological research involving the "manipulation of the genetic composition of micro-organisms" will continue in Britain, but in an atmosphere which takes account, chiefly through the laying down of proper laboratory procedures, of the possible attendant hazards. Stated like that, it all sounds eminently reasonable, but just what will happen next, and how hazard-proof can the eventual safety arrangements hope to be?

The working party finds that the possible benefits which may accrue from the continuation of such research are great—for example the 'mass production' of genes responsible for promoting the synthesis of insulin and the like. The report also describes the possible hazards (and says that there is always the possibility of these emerging in a completely unexpected way). Weighing the two sides up, the conclusion arrived at is that provided the precautions mentioned in the report are "taken by trained and disciplined workers, the potential hazards could be minimised, and the potential benefits would amply repay the trouble and expense which the precautions would involve". That is the report's thesis in a nutshell.

The real question is whether it is possible to impose from scratch, on scientists and technicians who have not been used to them, the disciplines of institutions that deal on a day-to-day basis with pathogenic organisms—those responsible for cholera and rabies, for example. It is certainly more difficult to persuade a whole laboratory that it must adopt new and time-consuming procedures than to convince an individual scientist, joining, say, a public health laboratory, of the necessity for all the precautions he finds there. The matter is made more difficult because the cholera vibrio is dangerous, period, whereas many of the genetic alterations brought about by cutting and joining pieces of bacterial DNA, or tampering with viruses, may or may not be harmful to humans. Alas, it would be all too human to pay only lip service to the best of precautionary arrangements, for that very reason. It is a salutary thought that even laboratories dealing with the smallpox virus are not immune from accidents of the type which caused the smallpox outbreak in London in 1973.

Containment procedures are, of course, important in the context of microbial genetics, but should they be the first line of defence as the working party suggests? The working party discusses several back-up procedures, including epidemiological studies on people carrying out work on genetic engineering of micro-organisms, and mentions ways in which, without prejudice to the science, at least some micro-organisms of interest could be made safe from the start. One possibility is to "dis-

arm" potentially dangerous plasmids or bacteria by altering their genetic composition a bit more so that, for example, useful but possibly unsafe strains of *Escherichia coli* could not survive in the human gut because the chemical environment or the temperature were wrong. (Those dealing with the most dangerous 'conventional' pathogens do not, of course, have that option open to them.) This is potentially a much more watertight approach than: a micro-organism may be able to survive in the human gut, it could be dangerous, so it should never get there.

Other ways of thinking have been canvassed—for example that the most dangerous experiments should be done in a few secure establishments—but where is the border line, what is a 'dangerous' experiment? In any case there would probably be scant support for the idea that a major growth area in the biological sciences should be confined to a few selected laboratories.

Much of the working party's report is, rightly, devoted to the risks to humans that might attend genetic manipulation of bacteria. But there is, as the report acknowledges, an "analogous risk" from similar work on animal and plant diseases. Here containment is very much the order of the day, is accepted as such by those involved, and in the case of some dangerous organisms is already backed up by legislation.

Assuming that the main thrust of Lord Ashby's report is accepted, what will happen next? The document is for discussion, and discussed it will be, especially at the conference at the end of this month arranged by Professor Paul Berg, one of the instigators of the National Academy of Sciences moratorium. The institution of a code of practice was recommended by the Ashby working party, but it was not within its brief to come up with chapter and verse. In Britain, there is already a body that might fit the bill—the Godber Committee, which is examining safety procedures in the laboratory use of dangerous pathogens following the 1973 smallpox outbreak. This working party is expected to report soon. (One of the suggestions made in the Ashby report is that a biological safety officer should be appointed wherever the experiments in question are to be carried out. That would not be sufficient, and in practice a system of inspection would be the only real way of keeping things up to scratch.)

Whatever happens, the Medical Research Council has stated that it will allow the research to go on, but accompanied by containment precautions of its own if nothing is imposed from above. The universities (through the University Grants Committee) have also taken the points made in the report and are examining in detail the situation as it affects them with a view to setting up their own rules. □



Immigrants at Lod airport. Photo Enka.

Coping with an exodus

The Soviet government's refusal to accept the conditions of the trade agreement with the United States has left in an equivocal position the Russian Jews waiting to leave their country. Meanwhile, Israel carries on with a reception programme which has handled 100,000 immigrants since 1970. Vera Rich reports from Tel Aviv.

THIS month, the 100,000th Jewish emigrant to leave the Soviet Union since 1970 arrived in Israel. In spite of Soviet claims that 98.5% of all Soviet Jews wishing to emigrate are allowed to do so, there is known to be a backlog of some 140,000 applicants still awaiting permission to leave. Although the effect on Jewish emigration of the recent Soviet rejection of the US-Soviet trade agreement cannot yet be gauged, there is no indication that it will lead to any official change of policy—indeed, a statement made by Colonel Alpachnikov of OVIR (the visa department of the Ministry of the Interior) on January 17, the day after the rejection of the agreement was announced, suggests that the policy will remain as before, one of frustrating delays and harassment, but with no absolute bar to emigration. The flow of Soviet Jews arriving in Israel may well slacken off for at least a time, but so far there are no signs of a total halt.

The problems of absorbing these immigrants into the Israeli economy are well known, and have become, indeed, the subject of a number of wry Israeli jokes ("What does one do with 50 professors of Russian philology?"). The

situation is aggravated by the fact that, on the one hand, some of the newcomers have a higher education and wish to continue to work in a learned profession, and, on the other hand, this higher education is sometimes so extremely specialised that even the smallest change of employment amounts virtually to a change of metier (one young engineer, at present going through the absorption process, quoted his first degree as having been in "Strength of Materials of the Aircraft Construction Industry"). And even those scientists who are able to be absorbed directly into Israel's higher education and research system may well find their practical problems only just beginning.

The problem, for a recently arrived scientist from the Soviet Union is largely one of adjusting his or her mental attitude from that of state-run, state-controlled research, to what has been described as the "anomic situation" of Israeli academic life. For the first time, they are faced with the problem of justifying their proposed research on an economic basis. It is not only the problem of what Israel can afford to pay for (some subjects such as

high energy particle physics which can only be tackled on a multinational or superpower budget, are clearly ruled out); they have to cope with a whole new system of funding projects and buying materials. Coming from a system where new graduates are directed into industry or research, they have no experience of such basic procedures as interviews and applications. Some are distressed by lack of 'status'—the new immigrant working in an Israeli academic institution has his salary paid for the first two years by the government, a situation which bypasses the current cut-backs and redundancies necessitated by Israel's economic situation, and also somewhat relieves the budget of the department concerned, releasing funds for equipping the immigrant scientist for his research. During these two years, however, he is essentially on probation and his appointment still awaits confirmation. Coming from the Soviet system, where the status of a scientist is extremely high, some scientists seem to find difficulty in accepting, even temporarily, a position in which they have a salary but no status.

It is easy, of course, to exaggerate the importance of such difficulties—and the comments of the scientists themselves must be treated with a certain discretion. Many have, after all, chosen to come to Israel since they feel that the Soviet system is not conducive to academic freedom. One physicist in particular, who, when interviewed about his reactions to working in Israel, took advantage of the opportunity to offer extensive criticism of the absorption process, and, indeed, of the Israeli economy and political system, concluded by saying that he loved living in Israel "for here I can criticise as much as I like." Many criticisms seem to arise from a sense of enthusiasm. Scientists settled in a job have anecdotes of friends awaiting absorption, who want "to use their resources and experience" for their new country. More organisation is needed, they urge. "Here, in Israel, all things go slowly."

To hasten the process, the Israeli government set up, at the end of 1973, a Centre for Absorption in Science, under the auspices of the Ministries of Labour, Absorption, and Finance, and of the *Sochnut* (Jewish Agency). It is staffed by seven 'professionals'—themselves scientists who felt that their real metier lay in administration rather than research—assisted by a small secretarial staff. Doctor Uri Horowitz, Director of the Centre, explains their task of that of "intervention to get the scientist into the centre of his own communications network", in other words, to familiarise him with the whole job-finding routine and to introduce him to the various contacts he will need in his professional

life. Although the centre does not deal exclusively with immigrants from the Soviet Union, they do, in the present circumstances, form the majority of its clients. The centre aims to promote the good of the scientist rather than the product, and, accordingly, the new immigrant is presented with a set of forms to fill in, in his own language, with questions structured according to what he expects in an enquiry of this kind—even if, in certain cases, the information is irrelevant to the needs of the centre. Having completed his forms, the immigrant is interviewed and questioned about the work he would like to do. He is then asked to write a feasibility study of his proposed project, with the emphasis on the part he himself would play in it. (It seems that some idealists have submitted projects which they feel would benefit Israeli science, but have forgotten to write in a job for themselves.) After completion of this study, the centre then discusses ways and means of fitting the scientist and his proposed project into the existing structure of Israeli science.

In general, the impression gained from interviews with scientists who have been absorbed is that remarkable efforts have been made to keep them within their same general discipline. Some of the cases of "change direction" quoted prove on investigation to be nothing more than change of employment. Thus the Kinneret research station (attached to the Technion) has absorbed five immigrants from the Soviet Union, only one of whom is a limnologist, the other four being physicists and chemists, who are, however, still working in their original fields, although now on the physics and chemistry of lake water. The need for 'retraining' often referred to may cover nothing more than the need of a computer expert to learn a new programming language. Some scientists arrived with a choice of interests, and have found their subsidiary interests in Russia can be their main interest in Israel. The main problems seem to arise with those who qualified not in one of the big state universities, but in a small Institute of Higher Education, where the subjects taught are extremely narrow and the graduate is, by western standards, more an applied technologist than a scientist. For some of these, retraining becomes, in effect, a chance to bring one's education up to full academic standard, and to advance from being, say, a computer technician, to a systems programmer.

Even for the best qualified scientists, there is, on arrival in Israel, generally a backlog of study to be made up. Under the current Soviet practice towards academic would-be emigrants, application for a visa is followed by a number of forms of harassment, including

dismissal from professional employment and the cutting off of one's telephone, which entails long and tedious journeys to arrange even the simplest details connected with one's hoped-for departure. If, as is often the case with scientists, the initial application is rejected, one may spend months or years as a "refusnik", with neither the opportunity nor the necessary time to keep up one's reading or think about one's own research. (The famous 'Sunday seminars' were started as an incentive to scientists in this position to attempt to keep up their work. On arrival in Israel, therefore, the scientist is not only faced with learning a new language, but with making up vast arrears of essential reading.

As far as possible, the universities do assist in the problem of learning Hebrew. The Ben-Gurion University (Beer Sheva) employs its immigrants on a kind of sandwich-course basis, half their time being devoted to studying Hebrew. Tel Aviv University, which has absorbed some 280 immigrants (including postgraduate students) has its own *ulpan* (Hebrew school for immigrants). The backlog of reading presents a greater problem—and is one cause, in the case of long term "refusniks", of scientists deciding not to continue in their chosen profession but to turn to some other field of activity like administration. Once an effort is made, however, and the reading started, the greater and more rapid availability of journals, and the chance to correspond freely with colleagues throughout the world, begin to show results and the information gap is rapidly reduced.

This freedom of correspondence, and the chance to work on one's own initiative, instead of as part of a closely controlled team subject to state direction, are seen by the scientists as the greatest benefits of their new academic life. The universities in their

turn find the immigrants from the Soviet Union a source of new vitality and expansion. The Pure Mathematics Department at Tel Aviv University was "virtually non-existent" until the wave of Soviet immigrants arrived; now it is acquiring a considerable reputation and the university is hoping to expand its Astronomy Department in a similar manner. The Ben-Gurion University, having absorbed seven Soviet pure mathematicians into senior teaching posts, is making tentative plans (subject to funding) to develop an Institute of Applied Mathematics that could take, initially, about 20 or 30 applied mathematicians. This university also offers considerable scope to Soviet scientists, especially those dealing with the more applied branches of physics and chemistry, in its raw materials programmes, and also to agronomists in its desert research programme. The vast scientific complexes of the Technion and the Weizmann Institute likewise still seem to offer considerable possibilities for absorbing further immigrants. The main problem remains that of funding—but Israel has a long history of foreign endowments for academic institutions and, in spite of economic difficulties, one feels that in the long run the money will be found.

Although an upper limit to absorption must at some time be reached—if only when the whole state of Israel is transformed into one vast academic campus, coterminous with the state frontiers, with the entire population either engaged in academic research or providing the ancillary services to keep the academics alive (the "think-tank of the western world", as one scientist described it)—that time has not yet come. And in the meantime, Israel provides a fascinating case study of the problems of transition from state-controlled research to one based essentially on personal initiative. □

Immigration officials check the papers of Viktor Polski, on arrival in Israel.



international news

SSTs: it's an ill wind . . .

by Colin Norman, Washington

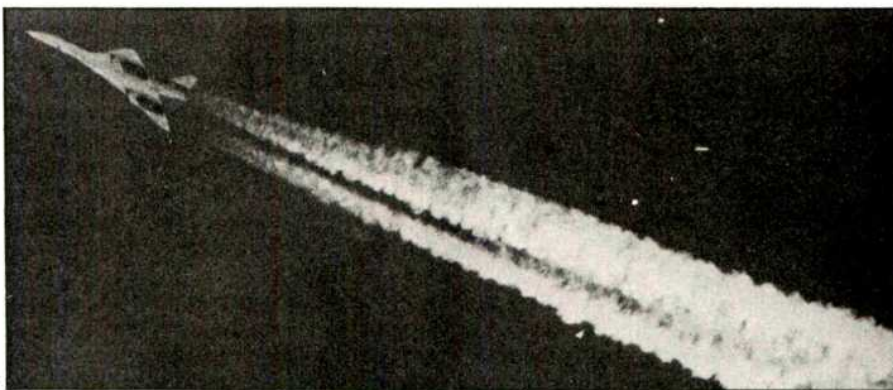
THE United States Department of Transportation last week made public the results of a three-year investigation (costing \$20 million) of the likely environmental effects of supersonic aircraft flying in the stratosphere. The event was a mixed blessing for the financially beleaguered Anglo-French Concorde project.

First, the study reached the conclusion that the present number of supersonic passenger aircraft (SSTs), together with those now scheduled to enter service, would cause climatic changes which will be too small even to measure. But that conclusion comes as no surprise since sales of Concorde have been so dismal that a maximum of only 30 SSTs are expected to be flying by the late 1970s (about 16 Concorde and 14 Soviet TU-144s)—a number which is unlikely to represent a threat to anything except Britain's balance of payments.

More ominous news from Concorde's point of view is that the study has essentially confirmed the validity of the much publicised theory that a large fleet of SSTs, equipped with present-day engines, could damage a layer of ozone in the upper atmosphere which shields the Earth from ultraviolet radiation. Consequently, if the size of the SST fleet increases significantly, serious environmental damage could result unless the aircraft are fitted with cleaner engines, the study indicates. The argument about the effect of SSTs on the environment is, therefore, unlikely to be stilled by this report.

Called the Climatic Impact Assessment Project (CIAP), the study was ordered by Congress after fears about the effect of SSTs on the ozone layer had played a key role in the Senate's momentous decision in 1971 to terminate development of an American SST. The basis for those fears has been hotly debated ever since.

The theory, put forward in 1970 chiefly by Dr Harold Johnston of the University of California, was that oxides of nitrogen, spewed into the stratosphere by jet engine exhausts, would upset the delicate chemical equilibrium of the ozone layer, destroy a part of it, and cause an increase in the amount



Concorde, accelerating at 35,000 feet

of short-wavelength ultraviolet radiation reaching the Earth's surface. One consequence would be to increase the incidence of skin cancer—an emotive topic if ever there was one.

Although it has turned out that Johnston's calculations (*Science*, 173, 517; 1971) overestimated the effect of SSTs on the ozone layer, chiefly because they were based on erroneous government projections of the size and character of the SST fleet in the mid-1980s, the CIAP study has not refuted Johnston's theory.

The CIAP's report states that although there is still some uncertainty in parts of the calculations, a cause-effect relationship between operation of SSTs in the stratosphere and increased ultraviolet radiation reaching the Earth's surface has been firmly established. The extent to which a given increase in ultraviolet radiation will increase the incidence of skin cancer is more uncertain, the study suggests, but again, a link has been established.

Proceeding from the undisputed assumption that pollutants injected into the stratosphere can stay there for periods of three years or more, the CIAP report argues that the problem is not confined solely to supersonic aircraft since some wide-bodied subsonic jet airliners now fly in the lower stratosphere.

But how great is the effect? According to calculations presented in the report, the fleet of subsonic jets now in operation, which includes about 7,000 aircraft operating in the lower stratosphere, is probably depleting the ozone layer by about 0.1% over the next ten years, and the 30 or so SSTs which will be flying in the next few years can be expected to add a further 0.1% depletion. Those effects are, however, much too small to be detected since the minimum change in ozone level that could be picked up by a global monitoring

system, operating daily for 10 years, is about 0.5%, the report notes.

According to Dr Alan J. Grobecker, Director of the CIAP, it would take about 125 Concorde-class SSTs flying in the stratosphere for between four and five hours a day to reduce the ozone layer at the minimum detectable rate. Grobecker pointed out in an interview last week, however, that if demand for SSTs picks up in the early 1980s, it is conceivable that 70 Concorde and a similar number of TU-144s could be in service by 1990, which would cause at least a measurable change in the ozone layer.

To put those figures in perspective, however, the study points out that the ozone layer is about three times thicker at the poles than it is at the equator, so that there is considerable natural geographic variation in ultraviolet flux reaching the Earth's surface. Moreover, the distribution of ozone at a given locality can fluctuate by up to 25% from day to day. Set against those natural variations, the change likely to be caused by the SST is relatively minor. But the study notes that a small change in the average amount of ultraviolet radiation around the world may result in a relatively large absolute number of skin cancers. And that is not a prospect which government regulators can take lightly.

Thus, if the size of the SST fleet increases, the report recommends that SST engines should be redesigned to reduce the emission of oxides of nitrogen. Studies conducted by NASA have indicated that emissions of oxides of nitrogen from Concorde-type engines could be reduced by a factor of six without sacrificing performance, but it would cost about \$50 million and take up to 15 years. A more radical engine redesign, to reduce emissions by a

factor of 10 or more, would take up to 25 years, the report notes.

"If it can be made clear, very soon, that such reduced emissions will be required of aircraft of all nations, and if design to reduce those emissions is incorporated promptly into ongoing engine development, ozone reduction could then be kept near the current reduction due to aircraft alone (less than 0.1%) . . . for fleets up to 4,000 747-class subsonics at 11 km (38,000 feet) or 1,000 at 13 km (43,000 feet), and for 150 Concorde/TU-144-class SSTs", the report calculates. Over the longer term and with the more radical engine redesign, "stratospheric air travel could expand as a function of demand without affecting the environment adversely, in keeping with deliberate calculable standards enforcement".

The key to those sanguine predictions is early development of international standards to force SST emissions to be lowered. But, given the tortuous history of attempts to regulate emissions from automobiles in the United States, that will be no easy task. Nevertheless, the report argues that "the process of establishing and meeting standards should start now because of the long lead times involved".

As for the biological effects of increased ultraviolet radiation resulting from the operation of SSTs in the stratosphere, the CIAP report states that the percentage increase in ultraviolet flux is about twice the percentage decrease in the lower ozone layer. Thus, the smallest detectable depletion of the ozone layer—0.5%—would increase ultraviolet flux by about 1%. Though the effect of such an increase on the incidence of skin cancer is not readily calculated, the report notes that an upper level effect can be calculated by postulating that ultraviolet radiation plays the only role in development of non-melanomic skin cancer. An increase of 1% in ultraviolet flux would therefore cause an increase of 1% in the number of skin cancers.

In addition to those suggestions, the CIAP report also notes that another possibly serious problem with a large number of SSTs is that sulphur compounds in jet fuel could end up as solid particles of sulphuric acid floating in the stratosphere. The effect of that would be to filter out some of the solar energy reaching the Earth, which in turn could reduce the mean global temperature. Although it would take a very large fleet of SSTs to produce a detectable effect, the report notes that a difference of only a fraction of a degree would cost hundreds of millions of dollars in crop losses. The report therefore suggests that consideration be given to regulations to force high flying aircraft to use low-sulphur fuels. □

India's space programme

from Narender K. Sehgal, Jullundur

INDIA'S modest space programme is facing rough weather. Until about two to three years ago, things were going fairly well and were not too far behind schedule. Then generally difficult economic conditions, coupled with inflation and rising costs, began to take their toll. Funds have since been scarce and slow in coming, so important projects are running late, and some have had to be curtailed or postponed. Others face an uncertain future as decisions on them are kept pending.

Space research formally began in India with the creation of INCOSPAR (the Indian National Committee for Space Research) in the Department of Atomic Energy (DAE) in 1962. The programme started off with the launchings of sounding rockets from the Thumba Equatorial Rocket Launching Station near Trivandrum, mainly for meteorological and ionospheric investigations in the upper atmosphere. Following administrative and organisational changes over the years, ISRO (the Indian Space Research Organisation), a body under the Department of Space, took over responsibility for all matters relating to the country's space programme in 1969.

From small beginnings in 1963, when the Thumba station became operational, the programme has grown steadily and now covers a whole range of activities relating to rockets and satellites. The Space Science and Technology Centre (SSTC) near Thumba is the research and development unit of ISRO and is responsible for (1) work on systems and their components required in space research; (2) carrying out pilot production of equipment resulting from such work; and (3) developing an indigenous satellite launch capability not only linked to scientific exploration but also to communications, meteorology and remote sensing.

Besides the Thumba station and the SSTC, several other establishments are also engaged in work connected with different aspects of the research programme.

According to original plans, the high spot of India's space programme—the orbiting of an Indian satellite taken up and Indian rocket launched from Indian soil—should have been attained during 1974. By the end of 1972, however it had become quite clear that work on the project was running behind schedule and that the launch would not be possible before 1977. Apparently, the delay was mainly because of difficulties with the satellite

launch vehicle project, since in early 1973 it was announced that an Indian satellite would go into orbit in the middle of 1974 atop a Soviet rocket launched from within the Soviet Union. This was to be a much heavier payload than the one planned for an Indian launch. Though good progress has been reported from time to time on the satellite part of this latter project, the launch, first postponed to the end of 1974, has now been rescheduled for sometime during 1975. A satellite prototype was reported sometime ago to have undergone successful tests both at Bangalore and in the Soviet Union. The small delay in launching could be because of either or both of budgetary difficulties and technical reasons, though there are certainly some grounds for thinking that the reasons lie elsewhere.

Sources at the Department of Space and ISRO have flatly denied a report in a British Interplanetary Society journal that India had agreed to make port facilities available to Soviet recovery ships in the Indian Ocean in return for Soviet launching of India's first satellite (weighing about 300 kilograms).

It is acknowledged a request for port facilities, for space tracking and recovery ships, was in fact received from the Soviet Union sometime in 1973. But the sources maintain that no decision has yet been taken in this regard and that this has nothing to do with the Soviet Union's agreement to launch the satellite, the two being entirely separate matters. It is believed that Indian scientists have been invited to install research instruments in future Soviet space vehicles to the Moon and other planets, but the matter has yet to be discussed and finalised. A joint meeting of the space officials of the two countries is to take place in February this year for discussions on collaboration in space research.

Meanwhile, the Prime Minister recently told a consultative committee meeting that the original cost estimate of Rs15.6 crores sanctioned in 1973 for the satellite launch vehicle project would probably need to be escalated. "Apart from the increased cost, the special circumstances prevailing during the past two to three years have created difficulties in the way of speeding up the progress of the project", it was said. "Barring unforeseen problems, it is possible to achieve a satellite launch in 1978", the committee was informed.

The launch vehicle project is aimed at developing a four-stage rocket to fire a 40-kilogram satellite into orbit 400 kilometres up.

Full allocations of funds for the projects of the Department of Space have not been forthcoming, and this has resulted in an adjustment of priorities. □

Nuclear fuel plant mystery

by Colin Norman, Washington

A TECHNICIAN employed at a nuclear fuel fabrication plant in Oklahoma died in an automobile accident on November 13 last year when her car careered off a highway and struck a concrete culvert. The crash has brought national publicity to a bitter dispute over safety standards at the plant and sparked off a string of bizarre allegations which could reverberate for years.

When the accident occurred, the technician, Karen Silkwood, was on her way to meet an official of the Oil, Chemical and Atomic Workers' Union (OCAW) and a reporter from the *New York Times*, to discuss charges of lax safety precautions at the plant and allegations of faulty manufacture of plutonium fuel rods. Police investigators ruled out foul play and said she probably fell asleep at the wheel, but the union hired an automobile crash expert who concluded that she may have been forced off the road by another car. The matter is now under investigation by the FBI.

Although that event was mysterious enough, a series of reports by the Atomic Energy Commission (AEC) which investigated Miss Silkwood's charges have added yet another element of mystery to the affair.

Miss Silkwood and officials of OCAW launched the AEC investigations when they met AEC officials on September 27 last year to give details of 39 separate violations of safety regulations

which they alleged had taken place at the fuel fabrication plant. Operated by the Kerr-McGee Nuclear Corporation at Crescent, Oklahoma, the plant manufactures plutonium fuel rods for the Fast Flux Test Facility, an experimental breeder reactor installation being constructed in Washington State.

In the meantime, Miss Silkwood discovered on November 5 that her hands were contaminated with plutonium after she had been working with a glove box handling plutonium samples. She was decontaminated and found to be free from plutonium when she left the plant later that night. But when she reported for work the next morning, her hands, forearm, neck and face were again found to be contaminated with plutonium.

Then, on the morning of November 7, a nasal smear taken when she reported for work indicated a high level of plutonium contamination yet again, and urine samples she had collected over the previous two days were also found to contain significant levels of the substance. A subsequent check of her apartment led to the discovery of microscopic amounts of plutonium in the bathroom, kitchen and refrigerator, and her flatmate was also found to have two spots of low level contamination on her skin.

An investigation of those incidents was launched by the AEC immediately, but a report released by the commission last week simply adds to the confusion. The AEC investigators found, in short, that Miss Silkwood's contamination "probably did not result from an accident or incident within the plant",

and that the plutonium discovered in the urine samples "was not present in the urine when it was excreted". Finally, they suggested that Miss Silkwood "did ingest some plutonium on or about November 7, 1974".

The essence of those findings is that the AEC believes that Miss Silkwood may have contaminated her own skin, swallowed a tiny amount of plutonium and added plutonium to her urine samples. No explanation is offered of the reasons for taking such action, although others have speculated that she may simply have wanted to draw attention to the allegations of lax safety precautions at the plant. If so, self-contamination with plutonium seems an incredibly drastic step to take.

The Oil, Chemical and Atomic Workers' Union has sent the AEC's report to some scientists for review, on the basis of which Mr Anthony Mazzocchi, the union's Washington representative, said in a report to Kerr-McGee's union members last week that because Silkwood had a high respect for plutonium, "it is inconceivable to us that she would contaminate herself or her apartment deliberately". He suggested that "there is enough evidence to cause an investigation to be commenced into the possibility that Silkwood was intentionally contaminated by sources unknown to her", and called for the AEC to reopen its investigation.

Meanwhile, the AEC investigated the other 39 allegations of safety violations raised by Miss Silkwood at the September meeting, and came up with some evidence of inadequate precautions. The report, however, largely exonerated the plant management and the AEC termed most of the incidents minor.

A close reading of the report, however, indicates that even though some of the allegations may have been exaggerated, the AEC's findings still paint a fairly dismal picture. Take, for example, the allegation that there is a turnover of 60% of the work force at the plant each year. The claim, says the AEC is not substantiated because the actual turnover is 35%, which is still an amazingly high figure. Similarly, the AEC says that the allegation that 80% of the work force has less than two years of experience is incorrect. The actual figure is 62%.

● In Britain, the possible dangers of working with plutonium have been highlighted in the past few weeks by reports that the health records of workers at the British Nuclear Fuels Windscale works showed a small but significantly higher incidence of leukaemia than might be expected. Although the statistical validity of these results is still being debated, at least one union is undertaking an investigation into the situation. □

How do you get over the problem of calcium loss, particularly from the knee joints, during long space voyages? Recruit double amputees for astronauts, says Dr John M. Vogel, a specialist in nuclear medicine, working at the University of California at Davis.

As nobody has yet found a successful means of preventing calcium loss at the knee joints during periods of prolonged weightlessness, the Californian team is seriously investigating the next best thing: dispensing with knees altogether. As there are people around who have already lost their legs, why not make use of them as space pilots?

There could be many benefits from having legless astronauts. It might be possible, for example, to carry more of them in a given size of spacecraft than of the conventional biped astronauts employed so far. Double amputees would also presumably eat less food, and breathe less air; and, being more compact, would probably find the cramped living conditions of

a spacecraft more comfortable than would two-legged astronauts. And the use of artificial legs instead of real ones during space walks or sorties on to planetary surfaces may prove a positive advantage. Not requiring the protection of a space suit, such legs would be in their natural environment in space rather than being less useful than real legs, as they seem on Earth. And as extravehicular activity would be carried out largely with the help of machinery anyway, with planetary 'buggies' and the like, the fewer human limbs to cater for, the better.

Of course, this whole philosophy could get out of hand. One could imagine armless astronauts ultimately, or perhaps, as medical science advances, even more bizarre developments. The Californian team is making its study in preparation for a possible voyage to Mars, which would take about six months with present technology. So, double amputees watch out for the advertisements.

from Peter Goodwin

Two Israeli university presidents, Professor Yuval Ne'eman of Tel Aviv University and Professor Moshe Prywes of Ben-Gurion University of the Negev, have submitted their resignations in recent weeks. Each has his own reason: Ne'eman has accepted a top post in the Defence Ministry and Prywes has been put in charge of all Negev medical services (in addition to directing the operations of Ben-Gurion University's newly established medical school). But their decisions may also have been influenced by the increasing difficulties faced by all local university presidents, who are striving to meet costs that have risen by 30% to 40% in one year while income—from Israel's University Grants Committee and other sources—has remained virtually static or has even declined.

Their problems are further complicated by charges in the press that too much money is being devoted to higher education at a time when insufficient funds are available for primary and secondary schools. Much of the criticism is extraordinarily simplistic: comparisons are made, for example, between the sum spent on the education of a primary school child and the money spent on the training of a university student, without taking into account, among other things, the university's role as a research centre.

Also stemming from the current economic situation is the government request that Israeli professors either give up their sabbatical leave this year, or, alternatively, spend it at another local institution. Professor Amnon Rubinstein, of Tel Aviv University, believes the request is justified "in a year of military tension and economic crisis." His colleague at Tel Aviv University, Dr Benjamin Rulf, has, in contrast, received the idea very coolly. Rulf warns that overseas sabbaticals are an essential safeguard against "scientific degeneration."

It now seems that the professors who are supposed to go on sabbatical leave this year will be asked to remain in Israel, but that no binding regulations will be brought in.

Economies, meanwhile, are proceeding in other spheres. Classes at the Hebrew University, to give just one example, are ending this winter at 20.00 instead of 21.00 in order to cut down on lighting and heating costs. The university has also announced a freeze on the hiring of new personnel to fill vacant posts.

But such paring down around the edges will not be sufficient. Barring some drastic and unlikely increase in funds from either Israeli or overseas sources, large scale dismissals are inevitable. In fact, Tel Aviv University

has already stated that 400 posts will have to go—some through natural wastage, others through dismissals. Other universities are likely to follow suit.

● Research grants from overseas bodies continue to play an important role in keeping afloat Israeli institutions of higher learning. This is particularly true at the Weizmann Institute, whose scientists manage to obtain a good many grants in the face of fierce competition.

Among the recent grants to be announced at the Weizmann is one of \$146,000 from the National Cancer Institute of the US National Institutes of Health to Professor Leo Sachs for research into the carcinogenic properties of chemical compounds. It will help

some 1,500 or 1,600 newly granted patents (about the number issued in Israel, to both local and overseas applicants, in the course of a full year).

● An (unpatented) Israeli development which should be of interest overseas is "Help Pollution", a game of the Monopoly ilk now being used as a teaching aid in high school biology classes. Created at Bar-Ilan University by Dr Uri Yoel, it is won by the player who is most successful in his attempt to prevent pollution of the Sea of Galilee, the country's main fresh water reservoir, which is facing a very real pollution threat from the inflow of municipal sewage and agricultural fertilisers, as well as from the rubbish left around its shores.

As in Monopoly, players acquire property around the lake and then try to increase its value. To do so they must prevent pollution of their land and of the lake by, among other things, investing money in anti-pollution measures. Uncertainty is added by accidents, such as DDT seeping into the water.

Letter from Israel

from Nechemia Meyers

Sachs further develop his method, already adopted overseas, for testing suspect chemicals in tissue culture rather than in experimental animals.

German funds are as important a source of support of Weizmann Institute research as money from the USA, the traditional sugar-daddy of international science. The Minerva Fund, a subsidiary of the Max Planck Society, is alone providing DM5 million to finance 64 projects at the Weizmann Institute during 1975.

Industrially sponsored research at the Weizmann (and elsewhere) is growing, but still leaves much to be desired. Rehovot scientists, for example, have \$1.25 million in industrial research contracts, 70% from overseas companies and 30% from Israeli ones. Most agreements, negotiated by the Yeda Research and Development Company, involve plastics, chemicals or pharmaceuticals.

● Mr Issac Fleischmann, Director of the US Patent Office's Information Service, suggested during his recent visit that Israelis, in addition to developing their own processes and devices, should make a more vigorous effort to exploit those developed in other countries.

Fleischmann pointed out that relatively few patents have been issued in Israel, which means that hundreds of thousands of ideas protected elsewhere may be borrowed freely for use here. He found it strange that there are so few Israeli subscribers to the US Patent Office's *Official Gazette of Patents*, which each week carries a summary of

● While Israeli schools teach youngsters to protect their country's environment, Israel's national radio and television stations are playing a song which glorifies a man for inscribing his name on a public building. He is Baruch Jamili, a soldier in the 1948 War of Independence, who chose to write his name in tar on a water pumping station near Jerusalem. Seeing the name 25 years later, the song writer and singer Shlomo Artzi wrote "The Ballad of Baruch Jamili", which presented Jamili as a war hero and won the last Israel Song Festival.

The outraged Nature Reserves Association promptly issued a statement strongly attacking the song, which, it fears, will encourage non-heroes to inscribe their own names on public buildings.

Graffiti are not a new problem here, as witnessed by the inscriptions that have been left all over the area for untold centuries. But the signature of a Crusader knight on the wall of a dining hall in Sinai's Santa Katerina Monastery is history whereas a signature added by a contemporary visitor is regarded as vandalism.

A unique attempt to let the graffiti artists express themselves, and yet safeguard the environment, is being made at a scenic canyon in the Negev desert, near the Red Sea port of Eilat. Taking a positive approach, the local authorities have set aside a special slab of rock where visitors are invited to carve or write their names.

They are politely requested to leave the rest of the canyon untouched.

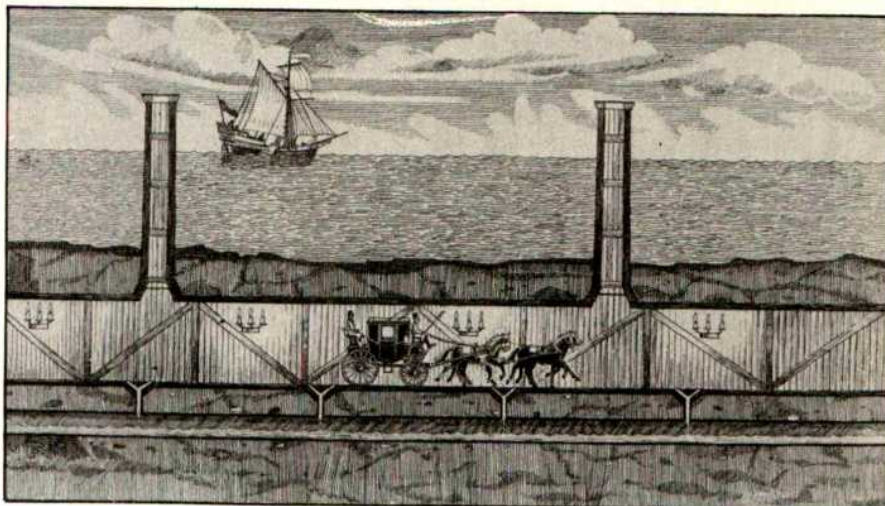
Corking the Chunnel

from Angela Croome

THERE have been complaints that the cancellation of the British-French Channel Tunnel Project last week by Anthony Crosland, Secretary of State for the Environment, was both hasty and premature. But this seems a point of view that is difficult to justify for a scheme that has been in the pipeline (as it were) for well over a hundred years. Indeed, a look at the 'on/off' history of this latest Chunnel plan (going back to the 1962 White Paper *Proposals for a Fixed Channel Link*) it is remarkable that the scheme survived the rejection in 1968 by both the British and French governments of the industrial proposals they had called for the year before. Rather than projecting an image of the 'white heat of technology', which was the watchword of the then Wilson government, the plan always had a gaslit and fustian air about it.

Tunnelling was above all a nineteenth century enthusiasm. It was indeed a technological innovation then—an extension of the techniques used in mining deeper and in some cases (as in Cornwall) under the sea. The Cornish engineer, Richard Trevithick, was called in to complete the first Thames tunnel (between Rotherhithe and Limehouse) in the early 1880s. With the aid of picked gangs of stout Cornish miners accustomed to working in a crouched position and in unbelievably horrible conditions he would certainly have succeeded had the money not finally run out only 200 feet from the far end. There is no trace of Trevithick's tunnel today. It took the Brunels, father and son, another 40 years to bring off the first Rotherhithe tunnel for wheeled traffic (now used by the London Underground). There were several cave-ins and a number of miners also died from the "tunnel disease"—a lung complaint attributed to the poisonous air in the workings. Many technological advances were made in completing "The Thames Tunnel" as the Brunel venture came to be called, including the special shield designed by Isambard Kingdom Brunel on the system of the burrowing shipworm *Teredo navalis* to hold the newly bored walls in place until lining was completed. But bridges increasingly superseded tunnels as a traffic link as the century progressed. And it is hard to see that there is any comparison at all in convenience, flexibility and 'passenger satisfaction'. The prospect of travelling not merely underground but underwater for the best part of an hour is a nightmarish prospect for many people.

Despite the long gestation period of



To Paris by carriage (with a gaslit and fustian air).

the Chunnel scheme, and the various assessments and reappraisals, the Cairncross review, the revised rail-link proposals and so forth, there has never been the sense of a real solution to the cross-channel traffic problem but rather a look of amateurish gimmickry. Has a serious study ever been made of a cross-channel bridge? Would it really be more vulnerable than a tunnel? Some years ago Desmond King-Hele put forward the virtues of a channel dam—and not entirely as a *jeu d'esprit*.

France is better linked to Britain than is any other European country. Nor can there be any question that another link with the capacity of the present channel ferries concentrated into the south-east tip of Britain would increase the congestion of the most crowded part of the country. There is considerable resentment already—in Belgium for instance—that so much freight has to be routed through France, picking up an additional and particularly swingeing tariff on the way. This has also put up the price of the goods in Britain.

There seems an excellent case for new links to the continent from the east coast of Britain and the more westerly of the south coast ports, thus better distributing traffic and avoiding the London bottleneck, and also bringing welcome growth to the less industrialised regions. This need not be motorised traffic either—whole trains travel by ferry in Scandinavia. The south coast port authorities have already indicated that they could handle twice the present traffic, which corresponds closely with the amount predicted for the tunnel at its planned opening date in 1981.

The potential of the hovercraft link seems to have been ignored in the predictions and calculations. Yet this type of ferry (using the current SRN-4, a 350-ton vehicle) takes only 30 minutes to cross the same route as the pro-

jected Chunnel, the journey in which was expected to last nearer an hour. Five craft now carry 25% of all passenger traffic on this route. Docking requirements, and therefore land use, is minimal. Apart from their rapidity, they perhaps represent the most flexible type of sea link yet devised. It is paradoxical that while the French have felt obliged to cry "perfidious Albion" again over Crosland's tunnel decision the partly state-subsidised Bertin company is hurrying to complete a larger cross-channel hover-ferry, the Naviplane 500, which is expected to be at the trials stage in 1976 and no doubt in competition with the British service shortly after. That there are no plans to develop a larger version of the SRN-4, nor a successor, is a matter of major concern in the industry.

Which brings one back to the lack of a true overall plan for traffic and transport development in Britain. What happened to the comprehensive independent study commissioned by the Ministry of Technology in the mid-1960s?

There is no reason to doubt that modern technology could build a channel tunnel if the nation was prepared to spend enough on it. If a prestige gesture for a technologically advanced (and united) Europe is a worthy object, then a Wilson-D'Estaing bridge would be much more telling than a Macmillan-De Gaulle tube. If better communications and a good return for money is the point, then the Secretary of State for the Environment has done the right thing and not a moment too soon, though it will be important to follow up a sensible saving with a sensitive alternative solution or solutions. The one bit of advanced technology that one must regret will not now come to fruition is the project of certain citizens of Kent for a Great Channel Tunnel Cork—to plug the great channel tunnel's mouth when it was finished. □

correspondence

Neuberger on nutrition

SIR,—I have considered myself a nutritionist for nearly 40 years, and I doubt whether even in the 1930s I would have ignored the teachings of John Boyd Orr and Frederick Gowland Hopkins in order to accept the description of nutrition that forms the first sentence of the Neuberger report: "The science of human nutrition is mainly concerned with defining the optimum amounts of the constituents of food necessary to achieve or maintain health."

Nutrition, on the contrary, is to do with the whole relationship between man and what he eats, that is, it is to do with food: how food is produced; what determines which foods we eat and how much; what the constituents of food are; which of these the body requires and in what amounts; how these constituents are dealt with in the body and what functions they perform; what happens when the required amounts of the required constituents are not provided or are exceeded; what steps can be taken to avoid these differences between what is needed and what is consumed. Nutrition, therefore, has reference to economics, anthropology, sociology, demography and psychology, as well as to chemistry, biology, biochemistry and physiology. In particular, nutrition is the one science that can least afford to remain in the laboratory; it concerns every single human being, every single day of his life.

What enthusiasm the report shows is confined largely to its summary of existing knowledge of the biochemical aspects of nutrition, and much of its recommendations for further research are also in this area. There is only an occasional brief reference to nutrition as it affects the whole body and an even less frequent nod towards the need to know more about people's nutritional behaviour. This bias is really not good enough; the human body exists together with other human bodies in a social and cultural environment, and important as biochemistry is, it is as important—and possibly more so—to know what determines the diets of different people, in different groups, at different times. Certainly we need to know more about energy transformation in the body and the mechanisms that control body weight and body composition. But of more immediate relevance to problems of malnutrition is the search for answers to quite dif-

ferent questions: Why do some people find it easy to cure their obesity and others find it difficult? Why are so many of the obese so easily persuaded that they can solve their problem by eating Ryvita or yoghurt, or by swallowing slimming pills that contain nothing but aperients, or by going to expensive but quite ineffective slimming clinics?

In this country, and in other countries in the western world, there is a considerable and increasing demand for so-called health foods. What is it that makes so many people entirely ignore the knowledge so laboriously acquired by nutritional research in favour of the incorrect or misleading information that makes them buy brown sugar, brown bread, sea salt, honey and vitamin pills to ensure that they are adequately nourished, or that makes them believe that there is special virtue in brown eggs or in vegetables grown with compost rather than with chemical fertilisers? And why do we continue to act as if it is still true that adequate nutrition in the industrialised countries is largely a matter of economic circumstance—as if all that matters is that a family shall have an adequate income—when it has been demonstrated in the USA, for example, that there has been an increase in the proportion of families eating a poor diet at the same time as family incomes have increased? Again it is just not good enough for the nutritionist to ignore these views, held as they often are by sincere and highly intelligent people, however misguided. Even less is it good enough for us to smile a superior smile and tell such people—and tell each other with a wink—how stupid these views are.

It is because nutritionists know that such matters are important that research carried out in departments of nutrition today is concerned not only with the physiological and biochemical problems that the report concentrates on so heavily, but with broader subjects too: the factors that determine food choice, the influence of diet on behaviour and of behaviour on diet, the assessment of attitudes towards foods and nutrition, and the differences between what people think about food and what in fact they eat. In the university department that I know—that at Queen Elizabeth College, London—more than 300 research papers were published between the start of the degree course in nutrition in 1953 and my retirement from the Chair of

Nutrition in 1971.

The report deplores the "relative neglect of human nutrition" as a subject for research. I suggest that this neglect is, more than anything else, a consequence of the existence of a myth about what nutrition is. The myth is that nutritional science is mostly to do with "defining the optimum amounts of the constituents of food". It is the narrowness of this account of nutrition that has been largely responsible for the subject's failure to attract either the research support or the attention of young scientists to the extent that its interest and importance deserve. Although the report deplores these consequences, it perpetuates the myth that has helped to create them.

Yours faithfully,

JOHN YUDKIN

London NW3, UK

Asbestosis

SIR,—The best comment to be made on P. F. Holt's letter (January 10) about complacency over asbestosis is contained in the latest Annual Report of HM Chief Inspector of Factories, which says in relation to new cases of asbestosis and mesothelioma, and the latest figures, that they "reflect conditions in the past when, in the then state of knowledge, it could not be ascertained with any certainty what levels of air contamination by asbestos dust would endanger health". Later, describing a major long term medical environmental survey of asbestos workers, he says: "In Phase II of the survey, which embraced the larger manufacturers of asbestos products, 5,000 workers were medically examined and 700 representative personal samples were taken in the workers' breathing zones over 4-hour working periods. The results, which were most encouraging, showed that 92.6% of the dust counts taken were below the very stringent hygiene standard of two fibres/ml and reflect the very great efforts made by the major firms in the industry to improve standards of control."

Mr Holt's references to a London life of insulation from reality are wide of the mark. I work in Manchester, the geographical centre of the British asbestos industry, and spend much of my time in asbestos factories.

Yours faithfully,

W. P. HOWARD

The Asbestos Information Committee,
Manchester, UK

Manpower needs

SIR,—We read with interest your editorial (January 3) entitled "Manpower needs are not so easily estimated". We feel that, although you made some telling points, the bluntness of your critical edge and the scathing tone caused some important aspects of manpower problems and the role of the Manpower Services Commission (MSC) to be lost. The baby, to our dismay, went out with the bathwater.

First of all, the nature of the MSC and its relationship to the Department of Employment are by now well known to those with a practical interest in the field and are easily obtained. Perhaps the opportunity to publicise its role might have been taken by a note on the fly leaf of the report on the manpower implications of offshore oil and gas. It is a pity, then, that you got your facts wrong and referred to both the MSC and the Petroleum Industry Training Board as agencies of the Department of Employment. And in drawing attention to a misprint you tempted providence in the form of the printer's devil, who inserted another in the same line of your own piece.

So, please let all of us eliminate, as you say, the "emotive turn of phrase" and give some careful thought to the critical problems of manpower—of which the manpower to enable us to exploit offshore oil and gas is not the least.

Perhaps the wisest statement of your editorial was the title: manpower needs are not so easily estimated. The UK has devoted ludicrously small resources to studying and forming policy about manpower. It is, after all, perhaps our most critical national resource (and that statement is fast becoming a cliché) and yet, as you rightly point out, we have not had the major, in depth, study that is really necessary to provide a sound basis for action at all levels in respect of skills and manpower needed to exploit the North Sea.

In such a situation, the arrival of the MSC as an independent voice is an important step forward. Its role and composition was established by the Employment and Training Act 1973. The MSC is financed through the Secretary of State for Employment (but is not part of the Department) and through the Training Services Agency co-ordinates the operation of the Industrial Training Boards.

At this early stage, it is surely appropriate for the MSC to promote and publicise pieces of work, particularly those that summarise what is known. In the process, some ideas will come forward to be dismissed and some good work will be overlooked. All this will draw attention to the paucity of current knowledge.

The report that you criticise, to quote the introduction, attempts to provide an overview by drawing together the threads of previous studies. There is no guarantee that a literature search will provide the answers to any problem. In the circumstances, and in view of the public debate that is taking place, the MSC is surely right to publicise the findings, but not to endorse necessarily the conclusions reached. In that way we will all learn.

Perhaps we could also take the opportunity to make some comment on the problems of the supply of qualified manpower, drawing on some of our own research. By and large, the employing organisations which create demand for people, on the one hand, and the education and training institutions which create a supply, on the other, operate pretty well independently of each other. Any impression that we can forecast the demand for (for example) geologists and then set up the courses to provide them is so oversimplified as to be useless. Accurate forecasts are not possible in any case. We have to know more about the absorption of graduates into the economy—some emigrate, others go back into education, others move rapidly away from straight application of their basic discipline. More needs to be learned about how university courses might develop in the interests of the individual and society (taking a very long term view). Even if we knew precisely what we wanted, there remains the question of how the educational system and the people in it react or might be helped to react. Industry is notoriously weak at articulating a considered view (though you are right to say that many companies prefer to undertake specialist training of geologists themselves).

Thus, in regard to many aspects of manpower, we have an extraordinarily complex system working within a changing and unpredictable environment in which the components have very strong needs of their own.

Manpower needs are indeed not so easy to estimate. We trust we may see more encouragement of and support for those who are only now beginning to grapple with the problem.

Yours faithfully,

JOHN LAWRENCE
GRAHAM ATKINSON

*Institute of Manpower Studies,
London School of Economics*

International meetings

SIR,—The letter from S. Peller (December 13) is similar to so many of the past few years and raises again the recurring, vexing problem of the location of international conferences. Clearly

assurances by some governments of entry to all participants are meant by these governments to be honoured in the breach. Starting from the premises that international scientific meetings should, in fact, be open to all qualified scientists, and that the internal affairs of the host country should not be interfered with, I should like to make the following suggestion.

Each international union should set up a committee to investigate complaints from *bona fide* scientists who have been barred from a meeting. If the charges are verified, then the union would bar that country as host for other meetings for a period of five years, the restriction to apply even to meetings already scheduled. Thus any country would have the choice of playing politics or hosting international meetings, but not both. Of course, some measures would also have to be taken to prevent the scientific union itself from becoming political, as has happened to UNESCO.

Variants of this method could also be considered. Thus a country which does not allow its scientists free access to meetings, such as, for example, the Soviet Union, could be barred from having any of its scientists attend such meetings.

Yours faithfully,

SAM SILVERMAN
Lexington, Massachusetts 02173

Alien message

SIR,—If an alien civilisation should chance to receive the radio message broadcast to the stars from the Arecibo telescope (January 24) it would be fully justified in reversing the charge for any reply. I make this assertion because, to judge from your illustration, the original communication was itself reversed, vertically. Which is to say, sir, it was upside down (not that the concept of tops and bottoms means very much in terms of inter-galactic messages).

As the message stands, an alien listener might just about make out that we are trying to tell him something about a man who appears to be balancing on his head at the top of a Maypole.

I for one shall be surprised if anybody (from this galaxy or the next) troubles to reply to such blather, unless it is to tell us that his chaps go in for Morris dancing while walking on their hands.

Yours faithfully,

B. JOVE

Hove, UK

ED—Sorry; the message was garbled during transmission from the *Nature* office to the printing works. So much for the march of progress.

news and views

New nitrogen-fixing symbioses *in vitro*

from John Postgate

THE truism that rhizobia, the symbiotic nitrogen-fixing bacteria, never fix nitrogen away from a leguminous host plant may seem, to an outsider, more of a dogma than an experimentally based fact. But a convention of scientific publication is that one rarely publishes purely negative findings, so the absence of documentation in the scientific literature of this failure does not do justice to the effort which has been put into the question: the truth is that many workers throughout the world have attempted, without success, to obtain fixation by pure cultures of rhizobium *in vitro* using all sorts of plausible conditions, such as nitrogen starvation at low oxygen tensions, presence of plant extracts, glutamine mutants and so on. A report in 1971 (*Nature*, **232**, 173) in which research workers from the laboratories of Dupont de Nemours obtained nitrogen fixation in a tissue culture of callus from a soyabean root, infected with *Rhizobium japonicum*, was greeted with excitement as a step in the direction of simplifying this nitrogen-fixing association. Fixation, though slight, was apparently taking place without the nodulation, leghaemoglobin and bacteroid formation seemingly necessary in the intact plant.

Though confirmatory reports gradually appeared from other laboratories, some scientists remained sceptical. Ethylene formation from acetylene, a diagnostic reaction for the nitrogen fixation enzymes, was the criterion used in these experiments; but ethylene is a normal product of plant metabolism. Were the controls for endogenous ethy-

lene as reliable as they looked? In addition, persons working with such material know well that it is very difficult to rid callus cultures of bacteria completely and the contaminants which persist are often capable of nitrogen fixation alone. They also knew that it is difficult (because of the gummy nature of the colonies) to be wholly certain that cultures of rhizobium are pure. Finally, the critics knew that aerobic organisms—bacteria or plant cells—could scavenge oxygen from a micro-environment and enable anaerobes such as *Clostridium pasteurianum* to grow and fix nitrogen. Were the microbiological controls of the early experiments adequate? Certainly they were cursory as described in the earliest publications and sometimes even absent.

Considerations of this kind account for the admirable preoccupation with cultural purity noticeable in two important developments, reported simultaneously from Canada and Australia in this issue (pages 350 and 351). Both papers present evidence that rhizobia, at least of the slow-growing 'cowpea' type, can form associations *in vitro*, not only with callus from the leguminous plants with which they associate in nature, but also with callus from plants which do not normally nodulate and even with callus from quite unrelated plant groups.

Like the Dupont group, all the workers obtained their rhizobia from Dr Burton of Milwaukee. Child not only checked for contaminants carefully but used three different strains of rhizobium and callus from six different types of plant, thus diminishing the

possibility that he is handling artifacts of contamination; Scowcroft and Gibson have also checked their material microbiologically and, in addition, with isotopic $^{15}\text{N}_2$, thus completely disposing of any possibility of errors due to endogenous ethylene production by the plant material. So, accepting that their meticulous negative tests for contaminating nitrogen-fixing microbes were adequate, the reality of effective nitrogen-fixing associations between slow-growing rhizobia and plants as remote from legumes as tobacco or the monocotyledonous wheat seems established, at least *in vitro*. It is entirely consistent with these findings, as both papers acknowledge, that Trinic recently discovered a natural nitrogen-fixing association between a slow-growing rhizobium and a non-leguminous plant of the genus *Trema* (*Nature*, **244**, 459; 1971). But the new experiments go further than this: their design is such that the plant callus is grown on a membrane, and this lies on an agar surface which has been infected with rhizobia. By removing the membrane the plant material can be removed from at least a proportion of its associated rhizobia. Yet the rhizobia remaining after the plant material had been removed continued to fix nitrogen for several hours. For the first time, therefore, rhizobia have been induced to fix nitrogen away from the plant, though the ability does not persist on subculture.

Physiological studies made it clear several years ago that rhizobia are the sites of nitrogen fixation in the nodule. Whether or not they possess the genetic information necessary to form the enzyme system is still not certain, but it is clear that, to use their information, they need something from the plant. The new experiments suggest that the material needed is diffusible and that its effect lasts for several hours. In the short term, biochemists, plant physiologists and geneticists will await eagerly the characterisation of the diffusible factor and the understanding of its apparent role in the regulation of the nitrogen fixation genes of rhizobium. In the longer term, these studies are a useful step towards the establishment of nitrogen-fixing associations between rhizobia and all sorts of agriculturally important crops and forage plants.

Elm bark disease in Australia

from Allan Rosel and John French

THE smaller European elm bark beetle (*Scolytus multistriatus*) was discovered in 1974 to be attacking elms in and up to 123 miles from Melbourne. This beetle is one of the main carriers of the Dutch elm disease fungus (*Ceratocystis ulmi*). It has not previously been recorded in Australia although the number of emergence holes in the bark of infested elms suggests that the beetle has been established in Victoria for several years. The beetle has been

sought but as yet not found elsewhere in Australia. A survey is continuing.

Microbial examination of beetles and attacked trees has failed to reveal the presence of the Dutch elm disease fungus. Dr S. L. Wood of Brigham Young University, Utah believes that the disease, if present, would have been apparent by now. It seems that in Australia, as on the west coast of North America, the carrier is present but not the disease.

New steps in the formation of phage T4 heads and DNA packaging

from P. J. G. Butler

THE large bacteriophage T4 has a long history of interest and current developments are maintaining this standard. Since it was shown to have a DNA-containing head connected by a hollow tail to the fibres which are involved in the attachment to the host cell, interest has centred on the mechanism for the assembly of such a complex structure. As shown by the groups at Caltech and Geneva, it is particularly suitable for such study because of the ability of the incomplete fragments, which accumulate in cells infected with mutants in many of the assembly steps, to form complete phage either by *in vitro* complementation with the fragments from another suitable mutant (Edgar and Wood, *Proc. natn. Acad. Sci. U.S.A.*, **55**, 498; 1966) or by subsequent activation of the blocked step (for example by the removal of a temperature sensitive infection from a non-permissive temperature to a permissive one).

The assembly occurs as a series of subassembly steps, with the head, tail and tail fibres being assembled separately and then joined together, first the tail on to the DNA-containing head and finally the tail fibres added to the fibreless particle to give the complete phage (reviewed by Wood *et al.*, *Fedn. Proc.*, **27**, 1160; 1968). During the infection of a host cell, the incoming DNA is expelled from the head through the tubular tail without any requirement for an energy source. This has led to speculation that the DNA is packed into the head in a special way, as if under pressure, so that its release occurs like that of a spring. A highly condensed state for the DNA is also required for the total amount to be packed into the volume available.

Head assembly

Unlike a number of simpler viruses, the heads of T4 seem to be largely assembled before much of the DNA is inserted into the pre-existing shell (Luftig *et al.*, *J. molec. Biol.*, **57**, 555; 1971; Simon, *Proc. natn. Acad. Sci. U.S.A.*, **69**, 907; 1972), and recently some of the steps have been described. A hierarchy of partially characterised steps (Laemmli *et al.*, *J. molec. Biol.*, **49**, 99; 1970) leads to the formation of the first recognisable head structure, prohead I, with a sedimentation coefficient of about 400S, containing at least four protein species (P23, P20, P22 and IP III) but no DNA (Laemmli and Favre, *J. molec. Biol.*, **80**, 575; 1973). These particles are converted to prohead II (about 350S) by the cleavage of

the major protein (P23) to give P23*. This cleavage seems to require the action of two distinct phage proteins, P21 and P24, and failure of either of these leads to the accumulation of 'τ-particles' (Epstein *et al.*, *Cold Spring Harb. Symp. quant. Biol.*, **28**, 375; 1963; Laemmli *et al.*, *J. molec. Biol.*, **49**, 99; 1970) which are smaller than the normal heads.

Though the τ-particles produced in the absence of functional P21 seem to be completely aberrant and cannot be rescued to form normal heads (Laemmli and Johnson, *J. molec. Biol.*, **80**, 601; 1974), those formed without P24 can act as a subassembly for head formation, without being disrupted in the process (Bijlenga *et al.*, *Nature*, **249**, 825; 1974). Analysis of the proteins in these 24 τ-particles shows that they

contain uncleaved P23 and that this is converted to P23* upon activation of P24, and it thus seems probable that these τ-particles represent an intermediate in the pathway between proheads I and II (Bijlenga *et al.*, *J. supra-molec. Struct.*, **2**, 45; 1974).

The conversion of prohead II into prohead III (550S) again involves the action of two phage proteins, P16 and P17, and is the first step in which the proheads become associated with the replicative DNA and the DNA packaging starts. During this conversion of II to III the cleavage of two further proteins, P22 and IP III, begins. This process is finished during the final maturation of the prohead III into mature phage heads. This requires the action of yet another protein, P49, and goes in close parallel with the com-

The magnetic field of Jupiter

from D. Stannard

THE latest development in the exploration of the Solar System by spacecraft came with the arrival of the Pioneer 11 probe at Jupiter in early December. Following almost a year after Pioneer 10, its predecessor to the planet, Pioneer 11 swept within 0.6 planetary radii (R_J) of the Jovian surface before continuing on its way towards the planned encounter with Saturn in 1979. With remarkable promptness, Acuna and Ness present a preliminary interpretation of the Pioneer 11 measurements of the structure of the inner Jovian magnetic field in this issue of *Nature* (page 327).

Ground-based studies of the radio emission from Jupiter over the past twenty years have suggested a picture of the field structure which is basically that of a magnetic dipole, with the axis of the dipole inclined by just under 10° to the rotation axis of the planet. Asymmetries which are present in both the decametric (noise burst) and decimetric (synchrotron) radio emission suggest that there may well be fine structure associated with the dipole field at distances of between 1 and 2 R_J from the planet. In particular there is evidence of a localised field distortion close to the longitude of the magnetic pole in the Northern Hemisphere (Conway and Stannard, *Nature*, **239**, 142; 1972).

The magnetometer measurements from Pioneer 10 confirmed the essential dipole character of the main Jovian field at distances greater than the spacecraft's closest approach of 2.8 R_J . A better fit to the data in the inner field region was however obtained using a 'dual dipole' model, with a weak secondary dipole close to the position of the field anomaly indicated by the radio astronomy measurements (Smith *et al.*, *J. geophys. Res.*, **74**, 3501; 1974).

The close approach of Pioneer 11 to the Jovian surface has now enabled the structure of the inner field region to be studied in detail. As shown in Figure 3 of Acuna and Ness's paper, the field is indeed complex, and at least an octupole expansion is required to describe the data. Particularly prominent is the asymmetry in the pole strengths of the two hemispheres, with a field of 28 Gauss at the north magnetic pole, and only 16 Gauss at the south. The magnetic equator within 2 R_J is highly distorted and steeply inclined with respect to the zenographic equator. With this detailed information about the field structure it should now become possible to perform more reliable quantitative calculations on the radio astronomical data to enhance our knowledge of the physical processes in the Jovian magnetosphere.

pletion of the DNA packaging. While a large part of the cleaved IP III remains in the mature heads, as IP III*, most of the cleaved P22 is lost and only the 'internal peptides' remain in the mature heads (Showe and Black, *Nature new Biol.*, **242**, 70; 1973).

Laemmli and Favre had shown that prohead II contained less than 1% of the DNA content of full heads and obtained some evidence that the prohead III contained about 50% of the DNA. This estimate has now been confirmed by the incorporation of bromodeoxyuridine into the maturing prohead III particles when a temperature-sensitive P49 is activated by a temperature shift, with further protein synthesis inhibited (Laemmli *et al.*, *J. molec. Biol.*, **88**, 749; 1974). After isolation of the DNA from the mature phage it was cleaved at the sites of bromodeoxyuridine incorporation, by exposure to ultraviolet light, and then found to have been reduced to about half its intact molecular weight. Using a similar technique, these authors also confirmed that the 24 τ -particles (and, by presumption, prohead I) contained little DNA.

The problem of the length determination for the DNA packaged into a mature phage particle has been further investigated using the giant heads produced by exposure of infected cells to L-canavanine and then deinhibition of the DNA synthesis with arginine. After isolation of narrow length classes, of about 4x and 8x normal head lengths from T4 and 12x to 13x from T2, and very gentle lysis of the phage to give intact DNA, it has proved possible to measure the molecular weight of the largest DNA molecules in the giant heads (Uhlenhopp *et al.*, *J. molec. Biol.*, **89**, 689; 1974). Using the technique of viscoelastic relaxation, these authors determined not only the molecular weight of these large DNA molecules (up to 1.5×10^9 daltons) but also the fraction of the total DNA which they represent. Though the large molecular weights found were fully consistent with the hypothesis that the DNA is packaged and only cleaved once the preformed head is full, without the need for any other size determining mechanism, the presence of about 95% of the DNA in the largest heads in smaller pieces means that the evidence is not conclusive against some additional mechanism.

Host cell membrane

Another interesting area of the interaction of T4 with its host cell is the involvement of the host cell membrane in the assembly of the phage heads. It has been known for some time that the head proteins are associated with this membrane and that this association is mediated through the phage protein P31 (Laemmli *et al.*, *J. molec. Biol.*, **47**,

69; 1970), but also mutant *E. coli* have been isolated which although permitting phage absorption will not support virus production (reviewed by Wood *et al.*, *1st John Innes Symp.*, 25; 1972). In one particularly interesting 'host defective' mutant cell line it has been found that the phage heads made are apparently normal, yet they are not filled with DNA and the rate of phage DNA synthesis is abnormally low (Simon *et al.*, *Nature*, **252**, 451; 1974). Two phage mutants have been isolated, which are able to overcome this block, and one of them is again in protein P31. Further evidence that the blocking may be due to alteration of the host cell membrane comes from the observation that it could be overcome by very low concentrations of chelating agent, which have their primary effect on the

bacterial membrane.

These results suggest that the host cell membrane plays a major part in the phage DNA synthesis. It also seems to have another, and rather less obvious, function during the disruption of the host cell DNA which follows infection by T4. This occurs by a dispersal of the host nucleoid with the association of the DNA with the cell membrane and its subsequent hydrolysis. Two 'nuclear-disruption deficient' phage mutants have now been isolated, which fail to cause this association although the host DNA does still slowly disperse throughout the cell and is hydrolysed normally (Snustad and Conroy, *J. molec. Biol.*, **89**; 663; 1974; Snustad *et al.*, *J. molec. Biol.*, **89**, 675; 1974). After comparing the DNA hydrolysis and release in various endonuclease-deficient mutants

Reversals through time

from D. H. Tarling

SEVERAL recent letters to *Nature* show increasing interest in the possible causal relationship between changes in climate and changes in the intensity of the Earth's magnetic field. Such correlations, if substantiated, would explain the extinction of various marine microorganisms at times of geomagnetic polarity reversal as, at such times, the Earth's field drops to about one-fifth of its usual value. The occurrence of polarity transitions during geological time is therefore of biological and climatological interest as well as being critical to models for the generation and evolution of the geomagnetic field.

The difficulty in studying such polarity reversals is that they are brief, lasting for some 10^3 to 10^4 years, in contrast to constant polarity periods which persist for up to 50 million years. This brevity makes them difficult to study as sedimentation in the deep oceans is too slow and lava eruption is too spasmodic to permit detailed palaeomagnetic studies, except in rare situations such as where the changing magnetic vectors have been trapped during the slow cooling of a large intrusive body. Even so, the average features of reversals during the last 70 million years are fairly well known and the pioneer studies of van Zijl *et al.* showed that their properties are similar to those of a reversal some 160 million years old in South Africa (*Geophys. J. R. astr. Soc.*, **7**, 169-182, 1962).

Some 2,000 years before a change in direction, the geomagnetic field intensity decreases by 80%, after

which the direction takes about 3,000 years to reverse and is followed by 2,000 years during which the intensity returns to its usual value. It is unlikely that the time scales of individual transitions differ from such generalised figures by more than an order of magnitude and the sequence seems to be consistent. Similar changes with similar time scales also occur during geomagnetic 'events' when the geomagnetic pole seems to topple, but then returns to its previous polarity without a true reversal having occurred.

These short time scales indicate that reversals must result from short-lived geophysical processes at the core-mantle interface. The identification of generally similar features during a polarity reversal some 1,600 million years ago, as described by Bingham and Evans on page 332 of this issue, is critical to geomagnetic climatic and evolutionary models. But it also has implications for the evolution of the interior of the Earth as a whole. Since essentially identical processes seem to have been involved at the core-mantle interface in mid-Precambrian times the basic parameters of the Earth, such as the dimensions and composition of the core and lower mantle, cannot have changed significantly since then. Hence it seems unlikely that any substantial differentiation can have taken place within the mantle within the last 2,700 million years (since Archean times), and convection in the mantle since then would, therefore, have to have been shallow.

of both the wild type and the nuclear-disruption deficient phage, these authors put forward a model for the nuclear disruption process. They suggest that the host DNA becomes attached to the cell membrane at several hundred sites during a normal infection. In an uninfected cell it might be attached at about five sites—the origin and terminus of replication and a few replication forks—while in the nuclear-disruption deficient infected cells it is probably attached at fewer than ten sites. Surprisingly, in spite of this major change in the effect of the phage on the host cell DNA, no difference was detected in either the burst size or the growth rate of the mutants and the wild type.

These various results show that the growth and assembly of T4 is still a subject of considerable interest and give hope that some of the intriguing problems may be cleared up soon.

Poly(A) tales

from Tim Hunt

THE November issue of the *Proceedings of the Academy of Sciences of the USA* contains an elegant demonstration that the poly(A) tail of globin mRNA is unnecessary for its translation in cell-free protein synthesising systems. Sipple *et al.* (Columbia) (71, 4635; 1974) hybridised globin mRNA with oligo(dT) and then attacked the hybrid with RNase H, a nuclease that specifically digests the RNA strand of a DNA-RNA hybrid. After this treatment, the reaction mixture was passed over a mixed gel filtration and nitrocellulose column to remove the oligo(dT) and undigested hybrids. The mRNA that resulted was shown to be free of poly(A) by its failure to form dsRNA with ³H poly(U).

It would be hard for these authors to be sure that they had removed literally all the poly(A); the possibility that a few residues remain would not, I think, be excluded. Nevertheless, this method for removing the poly(A) has advantages over the use of polynucleotide phosphorylase or the 3' exonuclease from eukaryotic nuclei. These exonucleases are processive enzymes—they do not leave their substrate until it is completely digested; hence, in order to digest all the molecules in a message preparation a molar excess of enzyme must be added, which in turn means that it must be highly pure. In addition, it is hard to stop the enzyme from going past the poly(A) stretch into the structural mRNA. Lastly, the RNase H method has a universal application for the removal of specific pieces of RNA if the complementary piece of DNA is available.

So what happens when you remove poly(A) from mRNA? The answer is generally agreed to be almost nothing. As a template for globin synthesis in cell-free systems (wheat germ, Krebs II ascites extracts, Schreier and Staehelin) the deadenylated mRNA is just as active as its unassaulted parent. This conclusion has been reached before by Williamson *et al.* (*Biochemistry*, 13, 703; 1974), Bard *et al.* (*Cell*, 1, 101; 1974) and Soreq *et al.* (*J. molec. Biol.*, 88, 233; 1974), all of whom used processive exonucleases to remove the poly(A); Williamson *et al.* and Bard *et al.* clearly did some damage to the mRNA beyond removal of the poly(A), but Soreq *et al.* used highly purified enzyme under very restrictive conditions, and their data are extremely similar to those of Sipple *et al.*

They did notice that the activity of the deadenylated mRNA tailed off earlier than that of the native mRNA, although the effect is not dramatic. Sipple's group also report a lowered stability *in vitro*, but the cell-free system itself loses a lot of activity over the same period of time, and the decay of the message has curious linear kinetics which is unusual; normally mRNA decays exponentially. In any case, it may be dangerous to argue from cell-free experiments to the situation within living cells, and with this caution in mind, Soreq's group arranged for their mRNA to be injected into *Xenopus* oocytes (Huez *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, 71, 3143; 1974). They found that both the activity and the stability of the deadenylated mRNA were apparently impaired. I think there are problems in the interpretation of these kinds of experiment, although the experimental data are impressive. The trouble lies in knowing how to quantify the amount of protein formed after injection of mRNA, owing to uncertainties about the degree of penetration of label and its dilution with endogenous amino acids. Huez *et al.* take as their measure a comparison between radioactivity in the 'endogenous' proteins and that in the peak of globin from a gel filtration column. The tacit assumption that the rate of endogenous protein synthesis is constant is very hard to test, and may not be correct.

Interesting and pertinent studies of the stability of RNA after its injection into oocytes have been made by Allende, Allende and Firtel (*Cell*, 2, 189; 1974). They injected radioactive RNA and followed the fate of the label rather than the stability of the template activity of the mRNA. They found that the RNA was degraded at an appreciable rate, except in the cases of tRNA and poly(A) itself; however, the decay of mRNA followed biphasic kinetics. This apparently results from the interaction of mRNA with cellular com-

ponents, possibly ribosomes, since the stable fraction is not seen in the presence of puromycin. These are provocative findings in the light of the extreme stability of the translational capacity of injected globin mRNA (Gurdon, Lingrel and Marbaix, *J. molec. Biol.*, 89, 539; 1973) and also of the fact that oocytes contain respectable amounts of stored mRNA which survive from oogenesis to fertilisation (Adamson and Woodland, *J. molec. Biol.*, 88, 263; 1974). The mechanisms ensuring on the one hand the stability of these messages, and on the other their unmasking during development are quite obscure. There were certainly no indications in Allende *et al.*'s experiments that the poly(A) played a part in the stabilisation of the injected messages; it would be interesting to repeat these experiments with radioactive globin mRNA with and without its poly(A), not an easy thing to arrange except by iodine labelling, since very high specific activities are necessary.

Taken together, these experiments suggest that removing the poly(A) may reduce the stability of the mRNA in certain circumstances; but there is little evidence from studies on intact cells to support the hypothesis that a long stretch of poly(A) confers a long life on a message. Although the poly(A) gets shorter with age (Sheiness and Darnell, *Nature new Biol.*, 241, 265; 1973), it seems that old messages have a similar life expectancy to young ones in view of the well established exponential decay of mRNA (see for example Brandhorst and McConkey, *J. molec. Biol.*, 85, 451; 1974). The recent description of mRNA which lacks poly(A) in HeLa cells (Milcarek, Singer and Penman, *Cell*, 3, 1; 1974) includes data on its lifespan, and there are no indications of anything unusual about the non-adenylated species compared with the adenylated. The poly(A) of mRNA still seems to be more of a natural gift to the molecular biologists than an essential component of mRNA. Yet the refrain is always: 'It's there, so it must do something'.

Histamine and the brain

from a Correspondent

AT a meeting of the Collegium Internationale Neuropsychopharmacologicum in Paris last July, ideas about the meaning of histamine in the brain were brought together and aired (*J. Pharmac. (Paris)*, 5, Supp. 1, 69; 1974). From this airing, it emerged that histamine is present in the brain, although in smaller amounts than are its relatives, 5-hydroxytryptamine and noradrena-

line. The histamine is partly in mast cells, but partly also in nerve terminals. It is distributed unequally in the various regions of the brain, the region richest in histamine being the hypothalamus, within which different nuclei have widely different contents. The L-histidine decarboxylase that produces the neuronal histamine is also present and achieves a rapid rate of histamine synthesis, naturally followed by rapid breakdown. In experiments in which mechanical lesions were made in the medial forebrain bundle (an area associated with reward), a decrease of the histamine content and of its synthesising enzyme suggested that a tract of histaminergic fibres had been severed. All this evidence, much of which can also be found in a well-documented paper by M. Garbard and colleagues (*Science*, **186**, 833; 1974), encourages the belief that histamine should be added to the list of putative mediator or modulator substances involved in brain function.

One of the best ways of deciding whether a putative mediator is a really serious candidate is to study the effects of its specific antagonists. Such tests, with the classical anti-histamines, whose development was started by Daniel Bovet and colleagues before the second World War, have clearly indicated that histamine plays a part in anaphylactic bronchoconstriction in the guinea pig and in nasal and dermal allergy in man. This criterion also suggested that histamine might be involved in such central nervous activities as wakefulness and motion sickness, because conventional anti-histamine drugs have sedative, hypnotic and anti-emetic properties.

These anti-histamines, however, failed in several directions in which they were at first expected to act; for example they did not inhibit gastric secretion induced by histamine. This difficulty led to the recognition of H_1 and other (later called H_2) receptors (Ash and Schild, *Br. J. Pharmac. Chemother.*, **27**, 427; 1966) and to the development of antagonists for the H_1 receptor and agonists for both types of receptor that were selective to the point of specificity (Black, Duncan, Durant, Ganellin and Parsons, *Nature*, **236**, 385; 1972).

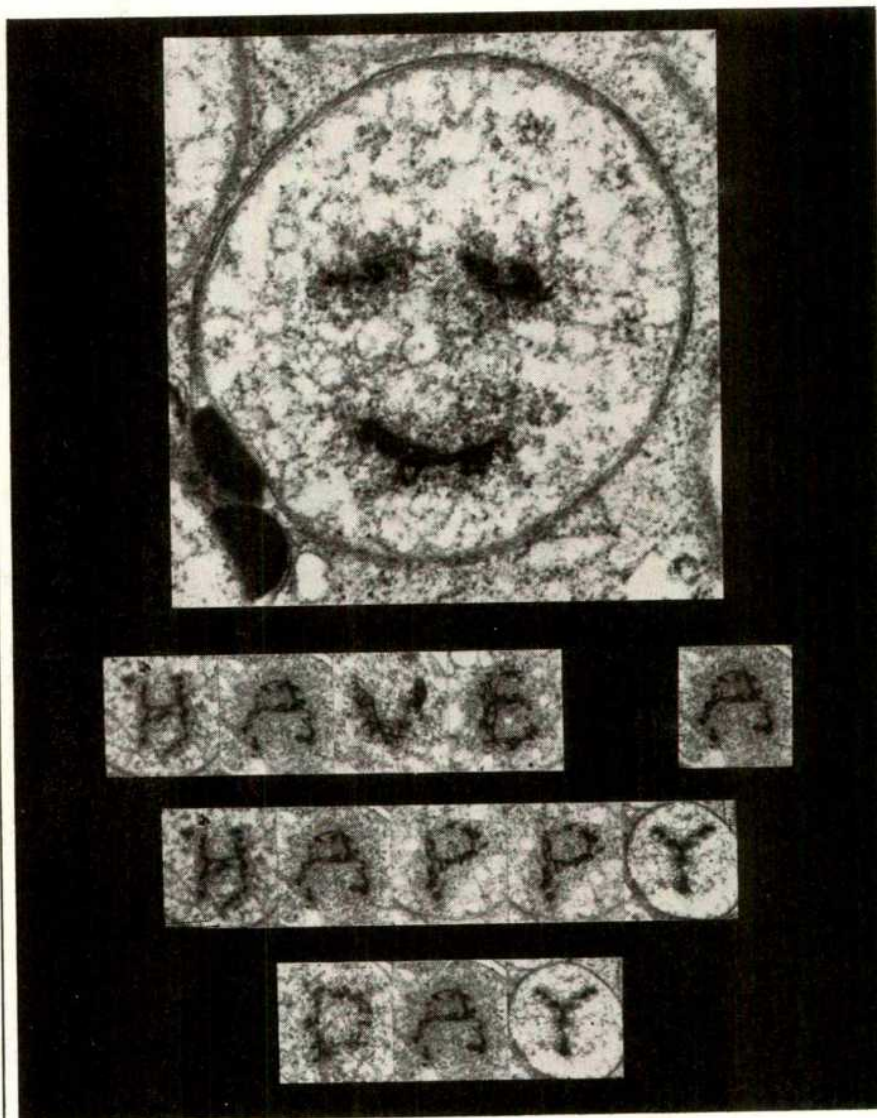
As so often happens, the availability of a specific receptor antagonist enables the action of the agonist to be clarified. In this issue of *Nature*, Baudry, Martres and Schwartz (page 362), report some insights gained from using specific H_1 and H_2 receptor antagonists (mepyramine and metiamide respectively) and an H_2 agonist (4-methylhistamine) to analyse the mechanism whereby histamine stimulates cyclic AMP formation in slices of guinea-pig cerebral cortex, an effect first observed

some years ago in rabbit cortex by Kakiuchi and Rall (*Molec. Pharmac.*, **4**, 379; 1968). The study of Baudry and colleagues shows that histamine acts on both H_1 and H_2 receptors to stimulate cyclic AMP formation, because it is not until both types of receptor are blocked that the effect of histamine is

obliterated. This finding fits with clinical experience, in that the central nervous effects of antihistamines of classical type are usually evident, but seldom intense. It would be interesting to know whether both H_1 and H_2 receptor blockers, given together, would produce profound sleep.

Cracking the genetic code is peanuts

from A. S. Craig and K. L. Giles



Genetic material of bacteroids in peanut root nodules, showing the only message de-coded to this date. Magnifications various.

DURING a study of the fine structure of root nodules from peanut (*Arachis hypogaea*) some features of considerable interest have been found. Routine electron microscope preparations of nodule tissue revealed plant cells full of large spherical bacteroids whose nuclear (genetic) material stained unusually heavily.

It did not pass our notice that the form of the genetic material in section often took on the appearance

of letters of the Roman alphabet. Armed with a knowledge of the genetic code one might have expected to find only the letters A, C, G and T. But so many were the letters found that it was possible to place several interpretations on them, one of which is shown. On these preliminary findings we are preparing a model of the genetic code based not so much on the idea of a macromolecular databank, as on a form of sophisticated semaphore.

- The use of specific H_1 and H_2 antagonists to analyse brain histamine action has been carried also into another phylum. Recently, Nahorski, Rogers and Smith (*Br. J. Pharmac.*, **52**, 121P; 1974) in experiments carried out in the cerebral hemispheres of the chick, *in vivo* and *in vitro*, found that H_2 receptor antagonists blocked histamine-induced cyclic AMP formation, whereas an H_1 antagonist was ineffective. Thus, the chick may differ from the guinea pig in that histamine receptors on its cortical adenyl cyclase may be largely of H_2 type, whereas in the guinea pig both types seem to be represented.

Glassy crystals

from Robert W. Cahn

In his stinging attack on Plato's political philosophy, Karl Popper (*The Open Society and Its Enemies*, chapter 3; Routledge, 1945) castigates Plato's "methodological essentialism", the delusion that to seek, by defining it, the essence of a thing can be a valid way of arriving at knowledge about the external world—including the world of social relations. Popper assures us that a true scientist always steers clear of questions which tempt him to definition-mongering, such as "What is an atom?" I am not at all sure that this is universally true: a passion for definitions sometimes provokes a scientist into a programme of experimentation which he might otherwise never have undertaken. The quest for the word provokes the deed.

These reflections are aroused by an unusual paper on the glassy state (Suga and Seki, *J. Non-Cryst. Solids*, **16**, 171; 1974). The authors, chemists at Osaka University, consider various ways of making non-crystalline solids other than by supercooling of a liquid, and ask whether it is plausible to call such solids "glasses". By itself, such a question deserves all the obloquy that Popper would direct upon it; but Suga and Seki went on to point out that the existence of a glass transition temperature is the crucial characteristic that has gradually come to be the defining criterion for glassiness. Thereupon they were moved to undertake a systematic study of glass transitions in a number of pure compounds nudged into non-crystalline states by cooling the melt, by condensing the vapour or by other more unorthodox means. In the course of these studies, performed in specially designed precision calorimeters (DTA apparatus), the authors discovered a number of anomalies: one compound might show more than one glass transition, and the concept of glassiness therefore required subdivision. It is rather as though St Thomas Aquinas

had conducted microscopic observations on the creatures cavorting on his needle-point and decided that it was necessary to distinguish archangels from mere angels.

Apart from a number of simple organic compounds such as isopentane, methanol, isopropylbenzene and cyclohexanol, the authors also examined a number of inorganic and organic high polymers, some inorganic materials (H_2O , $SnCl_4 \cdot 2H_2O$) and a range of compounds that form nematic or smectic (liquid crystal) phases. Thirty-eight materials were studied.

With regard to many of the simple compounds, it turned out that ordinary glass transitions, in the form of anomalies in the specific heat, turned up irrespective of whether the non-crystalline forms were prepared by supercooling a liquid, by condensation from the vapour or by rapid precipitation from solution. This answered the original question.

But three kinds of anomalous glass transitions were also found in some materials in addition to the ordinary transitions. Some compounds (for example, cyclohexanol, 2,3-dimethylbutane) have been known for some time to possess an intermediate structure at temperatures between full crystallinity and full liquidity, the so-called 'plastic crystal' state. The molecules are positionally ordered but orientationally disordered and mobile. This makes the molecules dynamically pseudo-spherical; because of this, they crystallise in face-centred cubic or body-centred cubic structures, which are unusually prone to plastic deformation: hence the name. Suga and Seki found that by rapid cooling they were able to freeze in this anomalous state, which they christened glassy crystal; on gradual reheating, the metastable glassy crystals passed through a 'glass transition' at which the metastable form transformed irreversibly into a fully ordered crystalline polymorph.

Another form of glassy crystal was found by supercooling of water, $SnCl_4 \cdot 2H_2O$ or $SnCl_4 \cdot 2D_2O$. Here the partial disorder was attributed to a freezing-in of the irregular positions of protons or deuterons; in this connection, the different transition temperatures for the last two compounds were of particular interest. There is a particularly full historical discussion of glassy ice—which, incidentally, has nothing whatever to do with the notorious polywater of a few years ago!

Finally, Suga and Seki examined a number of liquid crystals, in which there is orientational order, accompanied by positional disorder of molecules. It proved possible to 'freeze' this state by rapid cooling. The steady variation with temperature of the degree of molecular alignment, in the

'swarms' which are the liquid-crystal equivalents of ferromagnetic domain is thereby inhibited. When the glass liquid crystal, as the authors call the state, is heated through a transition the molecules regain their labile ability to vary their degree of alignment.

Two compounds—cyclohexene and ethanol—were found to possess two anomalous transitions each as well as a normal transition. The nature of the two anomalous glassy crystalline states is not yet known.

The authors document their findings not only by locating glass transition entropy with temperature: from these plots they are able to make further deductions about the nature of the change they have observed.

The concept of a glassy crystal was sometime a paradox, but now the tin gives it proof. Suga and Seki's paper is an impressive achievement which should stimulate many structural studies to confirm and extend their bold but sometimes tentative interpretations. Such studies may also have bearing on topochemical reactions (see the detailed and very instructive review by J. M. Thomas, *Phil. Trans. R. Soc.* **277**, 251; 1974). Topochemistry is concerned with the nature and kinetics of chemical reactions, including polymerisation reactions, between adjacent molecules in crystals, and such processes are bound to be modified if the state of positional and orientational order of molecules can be varied as then frozen into a particular pattern. Perhaps compounds can now be found in which internal reaction mechanism will change at glass transition temperatures. If this proves to be so, we shall need a new definition to denote the process: vitrochemistry, perhaps!

Behaviour in Europe

from a Correspondent

THE first European Neuroscience meeting will be held in Munich next September (for details see the advertisement on page vii). This meeting represents the culmination of several years' effort to promote collaboration between workers in different European countries and in different disciplines within the field of brain and behaviour research. The meeting is organised by the European Training Programme in Brain and Behaviour Research (ETP), a body whose history is of some interest.

In 1968, the Organisation for Economic Cooperation and Development inaugurated studies of three scientific fields: the criteria for selecting fields were that they should be multi-disciplinary, have a fast growth rate, have insufficient recognition, an

Climbing the technology tree

from Andrew Holmes-Siedle

THE Institute of Electronic and Electrical Engineers, one of the largest professional societies in the world, has turned some of its effort to helping those scientists and engineers faced with redundancy to plot their careers in such a way that they avoid the trap of 'irrelevance'. Its Technology Forecasting and Assessment Committee, whose original purpose had been to advise industry and government on planning, has now turned all its attention to an advanced form of career guidance. The committee has picked areas of application for which technology is being developed intensively and drawn

for each a 'technology tree' which relates these areas logically and lists the technologies involved in each.

To give an example, the Nuclear and Plasma Sciences Tree (Published in the IEEE Nuclear and Plasma Sciences Society Newsletter, October 1974) carries a limb entitled 'Radiation Effects' which contains eleven branches with 43 twigs, the subjects ranging from charge loss in capacitors to food preservation. To put it another way, the IEEE has identified some technological bandwagons and given us a few clues as to how we might climb on, the rest is up to us.

be likely to produce results both of scientific and practical importance within a decade. One of the fields chosen was Brain and Behaviour and in 1972 OECD published a report with this title prepared by two of its consultants, Dr Otto Wolthuys and Professor Stuart Sutherland. The report summarised the scientific status of the field and analysed a number of defects in its organisation in Europe including Britain. Among the defects mentioned were the lack of mobility of scientists, resulting in a slow flow of ideas and parochial journals, lack of communication between European countries, small laboratories with insufficient interaction between, for example, neurophysiologists and psychologists, and authoritarian departmental structures.

The OECD investigation led in 1970 to the setting up of ETP with a generous grant from the Max-Planck-Gesellschaft. It now receives contributions from Austria, Britain, France, Germany, Italy, Netherlands, and Switzerland and seven additional countries have representatives on the steering committee and participate in the programme. So far funds have been used for the following purposes:

- The training of young scientists in countries other than their own and usually in disciplines other than their primary subject
- Four highly successful winter schools for young scientists from all European countries—again with a multi-disciplinary emphasis
- 'Twinning' grants awarded to about 100 laboratories, enabling the members of each to travel to a sister laboratory abroad which is engaged on complementary work
- Grants enabling young scientists to attend conferences abroad organised by bodies other than ETP

In the five years of its existence ETP, operating on an insecure budget of about £50,000 a year, has done much to promote cooperation between workers in brain and behaviour in different European countries and in the different component disciplines. It is hoped that when the European Science Foundation starts functioning ETP will become affiliated, and that a more permanent flow of funds will be secured through international treaties. Unlike EMBO, ETP has set its heart against the founding of a large international laboratory since it is thought that the centralisation and bureaucracy involved are not in the best interests of the subject.

High-pass phonon filter

from P V E McClintock

SUPERFLUID 'He can be made to act as a tunable high-pass phonon filter, according to some recent experimental work at Nottingham University by Wyatt, Lockerbie and Sherlock (*Phys Rev Lett*, **33**, 1425, 1974).

Their experiment was performed below 0.1 K, where liquid 'He is an almost pure superfluid, that is, a fluid which can indulge in frictionless flow, or through which objects can move without any dissipation of their kinetic energy. The superfluid itself carries no entropy, but acts as the inert background or aether supporting a gas of phonons, which are the quanta of vibrational energy. These behave much like a collection of independent particles moving at the velocity of sound, and they carry all the thermal energy of the liquid.

The Nottingham experiment consisted, essentially, of injecting relatively

small numbers of very high energy phonons into the superfluid at temperatures below 0.1 K, investigating how they propagated through the liquid and, in particular, deducing whether they were able to decay. (Strictly, of course, the term 'temperature' is not applicable once the high energy phonons have been injected, because there then exists a non-equilibrium situation.)

Considerable interest attaches to whether or not a phonon of given initial energy is able to decay into two phonons of lower energy, since this yields direct information about the shape of the dispersion curve, that is, the relationship between a phonon's energy ϵ and its momentum p . It was long believed that for small ϵ the dispersion curve was linear, of the form $\epsilon = cp$ where c is the phonon velocity in the long wavelength limit, but that at higher ϵ the curve deviated below cp (negative dispersion), representing smaller phonon velocities. More recently, mounting evidence has apparently indicated that, although the dispersion curve is indeed linear at very small ϵ , there is an intermediate regime in which it deviates upwards (positive dispersion) before entering the negative dispersion region.

The precise shape of the dispersion curve is of considerable importance because of its profound influence on 3-phonon processes, that is, the decay of one phonon into two of lower energy or, conversely, the combination of two low energy phonons to create one high energy phonon. Such processes are of crucial importance in determining many of the properties of the liquid, such as the ultrasonic attenuation or the normal fluid viscosity, at temperatures near 1 K. It turns out that for 3-phonon processes it is only possible to satisfy simultaneously the conservation of energy and momentum in the presence of positive dispersion: the interactions cannot therefore occur at all where the dispersion is negative. Unfortunately, it is difficult to deduce the shape of the dispersion curve unambiguously from, for example, normal fluid viscosity measurements near 1 K. This is partly because phonons of a very wide range of energies are simultaneously present, but also because of complications arising from the existence at these relatively high temperatures of large numbers of rotons, which are another type of thermal excitation.

Hence the value of performing experiments at very much lower temperatures where the thermally excited 'background' phonons are so few that they can be neglected. The Nottingham group used a superconducting fluorometer to inject into the liquid pulses of phonons which all had energies larger than 2Δ (Δ being the energy gap of the superconductor), and detected

the phonons again after they had travelled several mm through the liquid with a superconducting tunnel junction, a device which is sensitive only to phonons of energy greater than 2Δ . Thus, the decay of an injected phonon would have yielded two phonons each of whose individual energies would have been too small to be seen by the detector, so that the observed attenuation of the phonon pulse could be regarded as a direct measure of the extent to which 3-phonon decays had occurred during the transit.

The main experimental finding was that the number of phonons reaching the detector depended strongly on pressure for pressures from 0 to 13.5 bar, but was almost pressure independent from 13.5 bar right up to the solidification pressure of 25 bar. The effect could not be due to changes in the number of high energy phonons generated in the fluorescer, since the performance of these devices is independent of pressure. There appeared to be two possible explanations: (1) some sort of dissipative effect occurring in the bulk of the liquid, attenuating all phonons, or (2) because of positive dispersion, the injected phonons might have been able to decay by 3-phonon processes, resulting in phonons of insufficient energy to stimulate the detector.

The first hypothesis was rapidly disposed of by altering the separation of the source and detector. The pressure dependence of the detector signal was quite independent of the separation, thus ruling out possible bulk attenuation effects.

The authors therefore conclude that the second hypothesis is the correct explanation. They suggest that there is a critical phonon energy ϵ_c below which a phonon decays rapidly, but above which a phonon will be stable and therefore able to travel long distances through the liquid. If ϵ_c decreases with increasing pressure, and if at 13.5 bar ϵ_c is equal to the minimum injected phonon energy of 2Δ , then the experimental data can all be understood. At higher pressures ϵ_c would become smaller than the lowest energy phonon from the fluorescer and the injected phonons would therefore all reach the detector without decaying, thus accounting for the observed pressure independence of the signal above 13.5 bar.

The scenario proposed by the authors is therefore as follows. When a pulse of phonons with an initially wide range of energies is injected into the superfluid, those phonons with energies less than ϵ_c decay almost immediately owing to the existence of positive dispersion, whereas those with higher energies, in the negative dispersion regime, propagate freely. Thus the liquid acts as a

high pass filter, passing only phonons of energy above ϵ_c . Because ϵ_c can be changed by altering the pressure, the filter is tunable and clearly has possible applications in the growing field of phonon spectroscopy.

This rather novel picture of liquid helium is certainly consistent with existing experimental information, but it is to be hoped that the authors will now carry out further checks on its veracity, in particular by repeating the experiments using detectors sensitive to a number of different phonon energies.

Structure and dynamics of spiral galaxies

from Vincent Icke and James Pringle

On Thursday, January 9, an informal discussion day at the Institute of Astronomy, Cambridge, brought together some 50 astronomers from various parts of the UK, to exchange news and views on the structure and dynamics of spiral galaxies.

BARRY MADORE (IOA, Cambridge) reviewed the situation nearest home, showing how the spiral structure of our Galaxy in the neighbourhood of the Sun can be determined by 'spiral tracers'. These are young, massive bright stars which, since star formation takes place predominantly in spiral arms, serve as 'standard candles' to delineate spiral structure out to distances of about 15,000 light years. Observing at Cerro Tololo, Madore has concentrated on the Northern Hemisphere which thus far has received only thin coverage. His results on the distances of classical Cepheids has extended the known nearby spiral pattern, but more observations are still required.

Passing to external galaxies, Greg Davidson (Jodrell Bank) and Darrell Emerson (Mullard Radio Astronomy Observatory, Cambridge) presented observations of the rotation speed and the neutral hydrogen distribution of the Andromeda Nebula M31. The dependence of the rotation speed on radial distance indicates the mass distribution in a galaxy. Davidson found that in the outer regions of M31, the rotation curve is flat, indicating a very large amount of mass in the 'halo' of this galaxy. The results, however, are not yet clear cut because the low resolution of the Jodrell Bank Mk I telescope tends to smear out and flatten the rotation curve. Emerson's observations of the disk showed ridges of condensed gas around the centre of M31,

forming rings or spiral arms (this galaxy is seen too edge on to enable one to say which of these two alternatives is the better). The velocity field, mapped by observing the Doppler shifts of the 21 cm hydrogen line, shows distinct non-circular motions, perhaps indicative of the motions which theorists predict for the gas condensations as spiralling density waves propagating through the disk. But here too the galactic inclination makes it impossible to distinguish between spiral and ring-like structure.

An alternative to density waves as the cause of spiral structure is the possibility that spiral arms are formed by galactic 'tides', generated by a previous close encounter of two galaxies. Observational evidence for such encounters was given by Anthony Winter and Geoffrey Cottrell (MRAO, Cambridge) who have respectively studied the pairs NGC9631-9656 and M81-NGC3077. An extensive neutral hydrogen complex is found to surround the spiral galaxies NGC9631-9656. From the velocity structure of the complex it is apparent that the gas actually links the two galaxies, the velocities in the gas match those of the galaxies and show a systematic trend from one to the other, thus linking them in 'velocity space'. Although the observations clearly show that the encounter profoundly disturbs each member of the pair, the influence on spiral structure is unknown because both galaxies are seen edge on. The pair M81-NGC3077 is more promising in this respect. M81 is one of the most beautiful two-armed spiral galaxies known. In this case, however, the evidence for tidal interaction is less strong. NGC3077 possesses a comma-shaped hydrogen extension, pointing towards M81, and this 'tail' may be a remnant of a hyperbolic encounter about 10^9 yr ago.



A hundred years ago

IN reference to the proposed Channel Tunnel between France and England, we may refer our readers to *NATURE*, vol 1, pp 160, 303, 631, and vol x, p 181, where the scientific bearings of the subject are pretty fully discussed. While on this matter we may state, on the authority of *La Nature*, that there has been in existence for some time in Spain an Inter-continental Railway Company, whose object is to connect Europe and Africa by a tunnel underneath the Straits of Gibraltar, the maximum depth of which is 819 metres from *Nature*, 11, 273 February 4, 1875.

To understand the large-scale motions in galaxies it is important to know how they interact with their surroundings. An aspect of this was studied by Roland Hunt (University of Oxford) who presented theoretical calculations of the effects which the infall of intergalactic gas would have on our Galaxy. The main consequences are a slow inward streaming of gas in the galactic plane and a peculiar relationship between the chemical composition of stars and their ages. The observational data are consistent with the no infall of gas at all, but an accretion rate of three solar masses per year is not excluded.

James Binney (University of Oxford) discussed a problem which has confronted theorists almost since the discovery of external galaxies, namely the origin of their considerable angular momentum content. In a universe which is initially homogeneous and isotropic (as ours appears to be) vorticity must somehow be generated. Binney has considered the creation of vorticity by the shock fronts which probably accompany the early stages of contraction of clusters of galaxies. It seems possible to account for the rotation of spiral galaxies, but the model may be rather sensitive to the shape of the shock fronts.

For simplicity, most theorists assume that the motions in a spiral galaxy can be satisfactorily simplified to motion in an infinitesimally thin disk. Douglas Heggie (IOA, Cambridge) presented some surprising results which indicate that one should be wary of such implications. He finds that, contrary to earlier expectations, the gravitational potential of a spiral wave can excite stars into resonant orbits perpendicular to the galactic plane. This drives stars far away from the plane, giving the disk a definite thickness. Resonance of the stellar orbits with the spiral pattern can then interfere with the propagation of spiral waves.

How a spiral wave might persist with a constant amplitude over the $\sim 10^{10}$ years' lifetime of a galaxy was discussed by Vincent Icke (IOA, Cambridge). He presented a wave equation in which energy losses and dispersive effects are counteracted by nonlinear terms, so that some wave solutions maintain the same amplitude indefinitely. Analogous equations in hydrodynamics and plasma physics exist which also exhibit these solitary waves or 'solutions'. Finding two-dimensional solutions, however, seems difficult.

Bernard Schutz (University College, Cardiff) presented calculations of the normal modes of instability of a galactic disk. In this type of analysis, the solution of Poisson's equation for the gravitational potential and of Vlasov's equation for the velocity distribution of the stars present formidable

obstacles. These were side-stepped by exploiting the thinness of the disk to approximate the gravitational potential by a polynomial series, and by treating the disk as a fluid. It is found that the fastest growing mode is a very open spiral, trailing with respect to the galactic rotation.

A computational approach to the origin of spiral structure was made by David Brownrigg (University of Reading), who used a cell-averaging technique to calculate the three-dimensional motion of 25,000 particles with mutual gravitational attraction. These particles represent Population I disk stars moving in a spherical potential corresponding to the average gravitational field of the Population II halo stars. The model seems to be a fair representation of a self-gravitating collisionless point-particle system. The model galaxy exhibits some impressive density-wave type spiral structure in its outermost parts.

Present thinking on the problem of galactic spiral structure was clearly reflected in the emphasis which the participants in the discussion put upon the observation of noncircular motion in single galaxies and of interacting galaxies, and in the preoccupation of most theorists with instabilities and wave propagation in differentially rotating disks. It seemed to be generally felt that progress in the understanding of spiral galaxies will be conditional upon the progress in these fields.

Identifying ancient forest

from Peter D Moore

THE climatic climax vegetation of England and Wales is generally considered to be deciduous forest, yet, as a result of human interference since prehistoric times, little, if any, woodland with an uninterrupted history now remains. It is natural, therefore, that conservationists should be concerned with devising methods by which fragments of ancient forest can be identified in order to protect them from further damage. The most useful criteria so far employed as indices of antiquity are the general species density of the habitat and the presence of species which are sensitive to disturbance.

Species richness is a concept which was touched upon recently in these columns when its relationship to time was considered (*Nature*, 252, 14, 1974). Southwood (*J anim Ecol*, 30, 1, 1960) demonstrated a positive correlation between subfossil abundance of British tree species and the number of associated insect species. He also demonstrated from Hawaiian data (*Proc*

Hawaiian ent Soc, 17, 299, 1960) that a general relationship exists in which the more abundant trees have higher numbers of associated insects. It is reasonable to expect, therefore, that assemblages of tree species which have occupied extensive and uninterrupted areas over considerable periods should support a rich insect fauna. It would seem reasonable also to extend this argument to associated plants and to use, for example, the richness of epiphytic bryophyte or lichen flora or of understorey herbs as an indication of old forest.

Rose and James (*Lichenologist*, 6, 1, 1974) have recently studied the lichen flora of the New Forest in southern England. This ancient Royal Hunting Forest contains wooded areas which have remained forested at least since mediaeval times and probably for even longer. The epiphytic lichen flora of the forest was found to be exceptionally rich, 259 taxa being recorded during the intensive six-year survey. Species density in some areas is as high as 160 km^{-2} which is a figure exceeded only in some extremely oceanic woodlands in the western extremities of Britain (Rose, in *The British Oak, Its History and Natural History*, edit by M G Morris, and F H Perring, 258, Classey, Farrington, 1974) where climatic factors are particularly suitable for epiphyte growth. It appears, therefore, that epiphyte species density can provide some measure of the historical continuity and lack of fragmentation of a forest.

The richness of the epiphyte flora of the New Forest cannot be explained simply in terms of the tree species available as hosts. Many epiphytes are selective with respect to host species, being particularly sensitive to the physical and chemical nature of the host's bark. Rose has determined the number of epiphytic lichens associated with various tree species and they fall in the order oak (303), ash (230), beech (194), elm (171) and sycamore (170). The New Forest contains mainly oak and beech, but the other epiphyte-rich tree species are rare or absent. Since oak/beech forest is common in southern Britain, the richness of the New Forest is best explained in terms of its historical continuity and also the low levels of atmospheric pollution experienced.

It is interesting to note in passing that the richness of Rose's epiphytic lichens is not related to the age or to the area of trees, or both, in the way that Southwood has demonstrated for insects. Some of our more ancient and widespread trees, for example, have a far poorer epiphyte flora than those mentioned above, such as birch (93), hazel (124) and alder (72). Southwood has suggested that an abundant tree is

more frequently encountered by an insect and that this assists the development of a feeding relationship. This process does not seem to have been generally operative in the case of the host/epiphyte situation during the post-glacial period in Britain.

Species density alone, therefore, may indicate antiquity in a forest, but it may also be misleading under certain conditions, for example, the increased microhabitat diversity which accompanies limited disturbance may, at least temporarily, inflate species density since it allows the invasion of species characteristic of unstable habitats. Also, local air pollution may deplete the epiphyte flora of even an ancient forest. A more hopeful approach to the assessment of past forest continuity is the use of species which are sensitive to disturbance. Rose has attempted this by constructing an "Index of Ecological Continuity" for woodlands based upon the occurrence of twenty selected lichen species which he regards as indicative of long periods of undisturbed woodland conditions. On this scale the New Forest has a rating of 100, very few other British woodlands have values of more than 70. Again, however, some woods with lengthy historical continuity could be underrated because of recent pollution, particularly in eastern Britain.

Peterken has recently proposed (*Biol Cons*, 6, 239, 1974) a similar system which he has used for the assessment of woodlands in Lincolnshire for conservation purposes. He selects flowering plants and ferns confined or almost confined to proved ancient ('primary') woodlands within an intensively surveyed study area and uses these as a yardstick for assessing woodlands over a wide region. His data demonstrate a positive correlation between the number of 'primary' woodland species recorded at a site and the area of the site. This suggests that richness in sensitive woodland understorey species is a function of undisturbed conditions in both temporal and spatial dimensions. As with the epiphytes, the oceanic conditions of western Britain are particularly favourable for the growth of woodland plants, hence some of the species indicative of antiquity in eastern Britain are more widespread in the west. Complications from air pollution, however, are likely to be less important than when using epiphytes as indicators, since understorey herbs are less sensitive.

Many of the plant species used by Rose and by Peterken as indicators of undisturbed situations grow slowly under conditions of low humidity and are poor in their dispersal capacity, thus spatial fragmentation of woodland presents them with problems of spread and survival. It is the presence of these

indicators, rather than the total number of species per unit area, which is likely to provide the clues for the detection and subsequent conservation of our dwindling fragments of ancient woodland.

Properties of plasmas

from A G Sitenko

The Second International Conference on Plasma Theory was held in Kiev from October 28 to November 1. The Conference was organized by the Institute for Theoretical Physics of the Academy of Sciences of the Ukrainian SSR and the Lebedev Physical Institute of the USSR Academy of Sciences and was sponsored by the International Union of Pure and Applied Physics.

CONTEMPORARY plasma theory is based largely on statistical methods—but there are different techniques for describing the properties of the statistical system. A G Sitenko proposed a detailed description of statistical and electrodynamical plasma properties, based on the consideration of fluctuations. Of fundamental importance in this description are the spectral correlation functions, which have simple analytical properties. In linear electrodynamics the fluctuation-dissipation theorem establishes a relation between fluctuations of different quantities and electrodynamic properties of the medium. That is why, once we know the fluctuation spectrum in plasmas, we can determine the electric plasma susceptibility by inversion of the fluctuation-dissipation theorem. The generalisation of this theorem for the non-linear case allows the description of non-linear electrodynamic plasma properties. Allowing for the non-linear wave interaction explains the saturation of the fluctuation level in non-equilibrium plasma under critical conditions. This level, corresponding to the stationary turbulent plasma state, can exceed the thermal level significantly. The kinetic equation for waves is derived, taking into account the interaction of waves with fluctuating fields in plasmas. To neglect this interaction (a procedure adopted in many papers) is not valid in nonequilibrium plasmas. In the stationary case the solution of the kinetic equation determines the fluctuation spectrum for the turbulent plasma state. Such an approach is very promising when considering the scattering and transformation of waves in plasmas.

V E Zakharov applied the inverse scattering problem method to non-

linear problems of plasma physics. He showed that a set of non-linear equations, which describes resonant interaction of three one-dimensional wave packets, may be solved exactly by the inverse problem method. The conservation laws are found and complete integrability of the equations is proved. In the case of decay resonant interaction between wavepackets the physical picture depends on the relative magnitudes of pumping rate and secondary waves' velocities. If the pumping rate is intermediate between the velocities of secondary waves, then the long packet of pumping decays practically completely when it collides even with arbitrarily small secondary wavepackets, but if the pumping rate is extreme, then the decay of the pumping wave is possible only when colliding with intense enough secondary wavepackets. The solutions for explosive instability are found, which describe the rise of local singularities in the limited wavepackets.

The question of turbulent plasma heating by beams of electrons and light was discussed by L I Rudakov on the basis of the soliton model of turbulence. The solitons may be formed as a result of a one-dimensional turbulent state modulation instability and are in fact sets of waves, trapped by the diminished plasma density domains, which, in turn, appear under the high-frequency field pressure. The formation of solitons is energetically advantageous, as it causes a decrease of the Langmuir quanta frequency, and the released energy promotes the formation of diminished density domains. As a result of collisions the solitons can decay or join. If the distances between the solitons exceed their sizes, then binary collisions play the main part. The electron changes its velocity when passing through the soliton. As a result of repeated collisions the electrons can increase their energy, thus changing the distribution function. This picture for the one-dimensional case is confirmed by the numerical integration of the non-linear equations of Langmuir turbulence. The joining of solitons can lead to the collapse phenomenon, that is, to dynamical accumulation of energy in certain regions of space. The existence of such a process, when special initial assumptions are fulfilled, is confirmed by numerical calculation. The interaction between particles and the collapsing formation causes the heating of the particles in the medium.

In conclusion, the conference gave participants a good chance to acquaint each other with investigations in plasma theory. The next International Conference on Plasma Theory will be organised by A Salam in 1976 at Trieste.

review articles

Unclocklike behaviour of biological clocks

Arthur T. Winfree*

The mechanisms which could underlie circadian rhythms fall naturally into groups with qualitatively different responses to disruption. Experiments designed to distinguish between these mechanisms seem to exclude all of them. It may be that multicellular organisms keep time, not by one 'clock' but by averaging many independent circadian oscillators.

"I hear the sound of time And yet I slew it, and
wiped my bloody sword upon its beard"¹

AN impressive diversity of organisms seems to have internalised the 24-h periodicity of this rotating planet. Exceptions include the prokaryotes, the bryophytes, most embryos and species evolved in soil, deep caves and the abyss²⁻⁶. But in most other creatures, body temperature, enzyme activity, leaf movement, neural firing, mitotic index or other convenient observables typically persist in regular up and down periods of about 24-h, even in ostensibly constant conditions²⁻⁶. These are called circadian rhythms to distinguish them from biological activities which persist in 24-h periodicity only so long as they are exposed to environmental cues at the same intervals. Brown and coworkers argue that even these circadian rhythms would stop or grossly change their period should the planet stop rotating, because they depend on the organisms sensing rhythmic fluctuations in subtle geophysical variables²⁻⁶. Although some quantities, such as the electrical charge of the atmosphere, fluctuate diurnally in synchrony around the whole planet⁷, many candidates for the role of covert driving rhythm are excluded by the persistence of circadian rhythms on a rotating platform at the south pole⁸. Though it is impossible in principle to exclude all hypothetical drivers experimentally, most workers since the 1930s⁹⁻¹¹ infer from the following generalisations that one ought instead to look for a mechanism of spontaneous oscillation within the organism.

The circadian period, though often precise to within an hour per month, seldom exactly matches the dominant periods of geophysical variables. The circadian period depends on temperature, typically drifts a few percent within one day and is chemically adjustable. Mutants of altered period exist. The phase of a circadian rhythm is labile with few exceptions (possibly including humans) it is permanently reset by a pulse of light or brief elevation of temperature within the physiological range or by temporarily withholding oxygen²⁻⁶.

Moreover, most workers seem to think in terms of an ultimately cellular mechanism, because organ and tissue-level interactions do not seem to be crucially involved.

Protozoa and unicellular algae exhibit typical circadian rhythms (for example of bioluminescence, motility, mitosis and surface antigenicity). The leaves of plants, bits of cut leaf or of cultured leaf callus independently support circadian rhythms of movement or respiration. Isolated mammalian organs and even disaggregated cells persist in

circadian variations of electrical activity, enzyme activity, respiration and so on²⁻⁶.

Attempts to pinpoint the cellular mechanism of such long-period rhythmicity have led to models emphasising cellular membranes¹⁰, cytoplasmic enzyme reactions¹¹⁻¹³, or sequential transcription of the nuclear message¹⁴⁻¹⁶. The diversity of action spectra for rephasing by light²⁻⁶ suggests a corresponding diversity of molecular mechanisms, at least at the photoreceptor end of 'the clock'.

Without wishing to deny the fascination of particular cases, I feel that a biophysical search for the unique mechanism of some universal clock is likely to be no more rewarding than earlier quixotic searches for the mechanism of 'homeostasis' or of 'excitability'. In such complex dynamical systems as living cells, stable steady-state operation can only be a result of the most intense selection¹⁷. Instabilities typically lead to spontaneous oscillation (the alternatives being some kind of semiperiodicity or unpredictable aperiodic fluctuations¹⁸). Thus the role of natural selection in adapting cells to a periodic milieu may be not to contrive and refine a timer so much as to weed out irregular fluctuations and oscillations of maladaptive period and to synchronise the rest. Such speculation discourages belief in a unique evolutionary ancestor of contemporary 'clocks'. It offers an interpretation of the curious facts that many circadian rhythms lack discernible selective value^{19,20} and that their evolution seldom if ever involved selective challenges during prolonged isolation from the environmental day/night cycle yet they cycle autonomously with precise period. It may be that the generic features of circadian rhythms stem more from convergent evolution and the common properties of complex dynamical systems than from a ubiquitous mechanism. For example, many of the peculiarities of the phase-resetting behaviour of circadian rhythms are also seen in a wide variety of electronic²¹, mechanical, and mathematical models²⁻⁶, and can be rationalised by simple topological principles without recourse either to such metaphorical models or to arguments from presumed selective advantage²².

What then can be learned by studying the phase shifts of biological rhythms? Such study leads to some 'unclocklike' aspects of whole-organism rhythmicity which were neglected so long as the "clock" metaphor, early introduced²³⁻²⁵ to emphasise the presumed significance of circadian rhythms for timing, biased investigators toward preoccupation with phase on a presumed rigid cycle. The encounter with unexpected and 'unclocklike' behaviour results from investigations into that timing role and the adaptive reasons for it.

Given the inevitable small discrepancy between the organism's circadian period and that of the environmental

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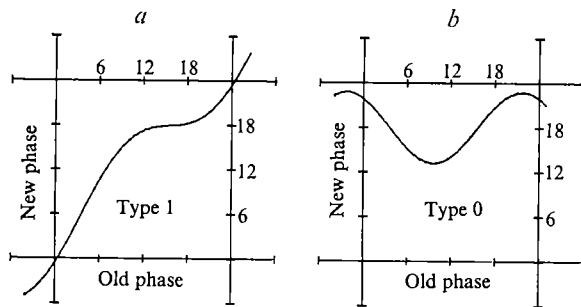


Fig 1 Old phase and new phase axes each extend through 1.5 cycles of 24 h. *a*, A type 1 resetting curve (typical of 'simple clock' mechanisms and weakly perturbed limit cycle mechanisms) rises through 1 cycle of new phase per cycle of old phase. *b*, A type 0 resetting curve (typical of strongly perturbed limit cycle mechanisms) shows no net drift along the new phase axis.

rhythms of food availability, predation, warmth, dryness and so on, an occasional phase correction seems necessary to maintain any preferred phase relation. The ultimate pacemaker and most reliable indicator of environmental phase is the rising and setting of the sun, that is, the light/dark cycle. With few exceptions, circadian rhythms are temporarily deranged by visible light: when normal rhythmicity recovers, it is found to be phase-shifted relative to an unexposed control. In *Drosophila*, for example, light as dim as starlight, if prolonged, damps out the rhythm²⁶, and a brief exposure at somewhat greater intensity changes the phase by as much as half a cycle before even 1% of the pigment molecules can have absorbed a photon²⁷. (The clock pigment of *Drosophila* is not in its eyes and is not rhodopsin (refs 28 and 29 and A. T. W. unpublished).) By altering the normal light cycle in agricultural fields with dim lights³⁰ or even a single nightly photoflash³¹, the seasonal adaptations of insect pests can be lethally disrupted, presumably through circadian rhythms involved in seasonal photoperiodism^{32,33}.

To ensure stable entrainment, the phase adjustment inflicted must vary according to the original phase at exposure. Measurements of this dependence have been carried out in many laboratories investigating the cell cycle³⁴, neurophysiological rhythms³⁵⁻³⁷ and circadian rhythms²⁻⁶. Particularly in the latter cases, certain peculiar regularities have emerged which seem hard to account for in terms of natural selection, but are typical of a broad class of dynamical systems (and atypical of others). Before introducing these data it will be useful to distinguish three traditions of thinking about the mechanism of biological rhythms.

Clocks and oscillators

In the first view, the mechanism underlying overt rhythmicity is called a 'clock' or, as in the cell cycle literature, a 'simple clock'^{38,39}. Its possible states can be arranged in a recurring sequence in which each state induces the next in a repeating cycle. The "state space" is a circle and there is no state which is not represented in that space. The clocks of home and industry are of this kind, the only variable quantity in the clock is the angular position of its meshed gears, and to get to a new position, it must pass forward or backward through all intermediate positions. Virus replication constitutes such a clock, the polymerase advancing round and round a circular genome at a rate limited by substrate availability, a similar model originally proposed for the cell cycle⁴⁰ is the basis of one conjecture about the circadian mechanism in eukaryotic cells^{14-16,41}.

Much of the literature implies that 'biological clocks' are clocks in this sense, that is, that the mechanism adheres to

a one-parameter sequence of states or 'subjective circadian times'. For example the widely adopted definitions 'phase' and 'phase shift'⁴² and many analyses of the process of rephasing⁴³⁻⁴⁵ implicitly or explicitly assume that the clock is always at some phase in its cycle, with phase changing at a rate determined by light intensity and current phase. Integrating this rate function as the phase changes during an exposure, one can calculate the new phase reached as a function of old phase for a given light intensity and duration⁴³⁻⁴⁵. 'Simple clock' mechanisms are distinguished by the smoothness of this rephasing curve (Fig 1*a*), its limitation to positive slopes and the fact that its mean slope is necessarily exactly 1 (as it is, for example, in response to a vanishingly brief stimulus, following which new phase = old phase)³⁹. We call this, by the mean slope of the rephasing curve, 'type 1' rephasing: the curve rises through one cycle of new phase in each cycle of old phase as it traverses to the right.

In contrast, Kalmus⁴⁶ and Mori⁴⁷, following Bonhoeffer's treatment of electrical rhythms in cells^{48,49}, were the first to emphasise limit-cycle oscillator models for circadian rhythms. In this view two or more variables (for example chemical concentrations) smoothly affect each other's rate of change in such a way that departures from equilibrium are not opposed. Either spontaneously, or once disturbed beyond a certain threshold distance from equilibrium, such a system quickly approaches a 'limit cycle' in which both quantities fluctuate regularly, at the same period but out of phase. Disturbed from this cycle, the system quickly returns to it, amplitude and waveform are subject to vigorous regulation. Regardless of their potential complexity (for example the number of variables involved), limit cycle mechanisms differ from 'simple clock' mechanisms in the possession of easily accessible states (including an equilibrium point) from which normal rhythmicity

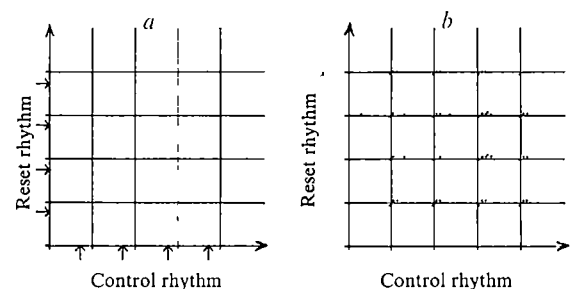


Fig 2 Cycles 4-8 of control rhythm event time are plotted against reset rhythm event time, both measured from stimulus time. Since event time (arrows in *a*) is the complement of phase at stimulus time, these axes are equivalent to backward old phase and new phase axes, respectively. *a*, Plot of a single experiment against its control, *b*, (arrows omitted) twelve experiments plotted, each with stimulus falling at a different phase in the control rhythm. Data represent centroids of *Drosophila* eclosion peaks, showing type 0 reset by 12,000 erg cm⁻² of blue light.

never develops, or does so at unpredictable phase after a unpredictable lag period^{49,50,51}. These phenomena are implicit in several mathematical metaphors employed by theorists of circadian rhythmicity^{52,53}. The rephasing curves of limit cycle mechanisms are not limited to positive slope and can have a mean slope of 1 or 0 (or any other integer if more than 2 variables are involved)³⁹. In 'type 0' rephasing, what goes up must come down before the curve passes one cycle of old phase to the right (Fig 1*b*).

And what about the ubiquitous relaxation oscillator model? According to this view, popular among biologists concerned with the cell cycle⁵⁴ and nerve activity⁵⁵ as well as circadian rhythmicity²⁻⁶, some photolabile metabolite accumulates until a threshold concentration is reached when it is all destroyed or a gradual destruction begins

which will be terminated at a lower threshold. Exposure to light or a temperature shock prematurely destroys the accumulated substance. Though such a mechanism may be viewed as a limiting case of a simple clock or limit cycle, it is conveniently treated separately. Its rephasing curves all have an abrupt jump whose magnitude increases with stimulus duration. Consequently 'average slope' is ambiguous.

Simple clock excluded experimentally

No circadian mechanism has been observed directly. But it is possible to measure the rephasing curves by monitoring any conveniently observable rhythm, on the assumption that a constant phase difference between an undisturbed control and the disturbed rhythm reflects the same phase difference between the circadian oscillators driving each rhythm (usually it is a few cycles before the disturbed rhythm recovers its normal period and the phase difference becomes constant). Taking the time of the rephasing stimulus as zero and plotting recurrent event times in the reset rhythm against control event times recurring at the same period, a square lattice of data points can be obtained (Fig 2a). Superimposing many experiments, in which the stimulus struck at all phases of the cycle, one obtains a square lattice of rephasing curves (Fig 2b). The plot is periodic along both time axes. Each unit cell of this 'time crystal' contains as many data points as there were experiments, typically arranged along a smooth rephasing curve that scans some integer number (including 0 as a possibility as in Fig 2b) of cycles vertically as it passes one cycle horizontally. This smoothness is the first fact. It eliminates relaxation-oscillator models, for which an abrupt jump in the rephasing curve is expected at every stimulus duration. (The "breakpoint" or "point of no return"³³ is often depicted in "response curves"—phase shift against original phase—by a data-less vertical dashed line spanning one cycle. This indicates the phase at which the predominant direction of short-term transients in the upset rhythm changes from delays to advances. This typically occurs where the eventual steady phase shift is close to a half cycle. There is no such jump in the rephasing curves.)

The second crucial fact is that every species shows type 1 rephasing in response to sufficiently faint stimuli. This is because a faintly reset rhythm is almost identical to its control, so plotting one against the other gives data points near the rising diagonal in the time crystal. Thirdly, curves with broad regions of negative slope and mean slope 0 are not uncommon and might even occur in all species, given a sufficiently potent stimulus. This eliminates 'simple clock' cycles in all species showing type 0 rephasing.

The fourth crucial fact emerges when the 'time crystal' is extended into three dimensions along a stimulus duration axis. The rephasing curves extend into wavy surfaces, but not separate surfaces as expected of a simple clock mechanism. One finds instead a single corkscrew-shaped surface winding around a vertical symmetry axis (Fig 3). At this isolated singular phase and duration of stimulus, the repeated surfaces connect together by tilting vertically, so that new phase is ambiguous. Following this stimulus, circadian rhythmicity is abolished or only recovers with random phase after an unpredictable lag period (Fig 4). The rephasing curves for stimuli exceeding this critical duration have mean slope 0, those for briefer stimuli have mean slope 1. This is the picture as it emerges in the only two species for which measurements have been carried out in sufficient detail, that is *Drosophila*^{22, 27, 39} and the succulent plant *Kalanchoe*^{36, 37}. Fragmentary evidence from a diversity of other circadian systems, perturbed by diverse rephasing stimuli, seems to fit the same pattern^{38, 39}. This is the pattern expected for limit-cycle mechanisms⁶⁰.

The existence of a singularity reveals states not on the 'clock's' cycle, and establishes the importance of some second and uncontrolled experimental variable, complementary to the 'phase' or 'subjective circadian time' which has apparently sufficed up to now for description of experiments and inference from their results. Perhaps some of

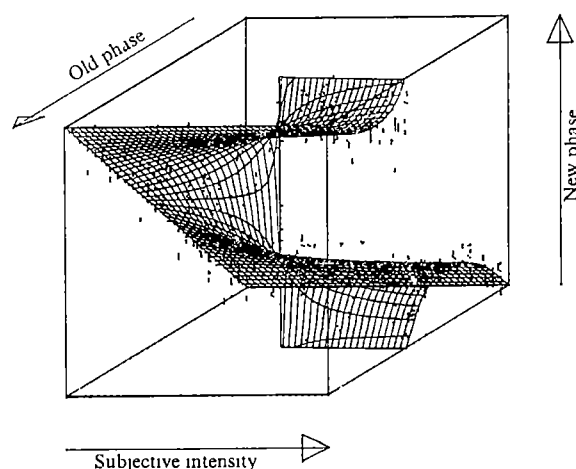


Fig 3 The curved surface depicts new phase (from 0 to 24 vertically) against old phase (from 0 at the rear to 24 at the front) and subjective stimulus intensity (from zero to the right) for the circadian eclosion rhythm of *Drosophila*, as calculated from the simplest oscillator model²⁷. Phase is recorded as h from eclosion peaks (extrapolated back in steps of 24 h) to stimulus time. The 1,327 data points represent eclosion peak centroids of clearly rhythmic populations ($R < 50$)⁶⁶, in experiments covering four cycles of old and new phase, all reduced to this one unit cell. Subjective stimulus intensity is exposure energy divided by an exponential dark-adaptation factor. Note that exposure during an eclosion peak (phase 0, the front and back edges) does not affect subsequent eclosion timing, and exposure midway between peaks at a critical intensity results in ambiguous phase (the vertical pole around which the surface and data climb). The data and surface in this unit cell would fit smoothly on to replicas above, below, in front, and behind.

those inferences could now be re-examined. A similar dilemma seems to be arising through Kauffman and Wille's application of similar experiments^{50, 61} to the cell cycle—the familiar 'simple clock'³⁸ and 'mitogen relaxation oscillator' interpretations³¹ of cellular control over mitosis provide no interpretation for a singularity or for type 0 rephasing.

Limit cycle excluded experimentally

In the singularity we are confronted with a first 'unclock-like' aspect of circadian chronometry. A second emerges from the attempt to pursue the idea of a quickly recovering limit-cycle mechanism through the entrainment of circadian rhythms by stimuli repeated at 24-h intervals.

Perkel *et al.*³⁵ carried out an elegant theoretical and empirical analysis of entrainment for pacemaker neurones exposed to regular synaptic input at millisecond intervals. This analysis is built on the assumption that the oscillator pops instantaneously from one phase to another of its cycle. The same assumption led to the same equations in Ottesen⁶², Pavlidis³³, and Pittendrigh's⁶³ analysis of circadian rhythmicity (hence the acronym 'POPP' model). The success of the POPP model for circadian rhythms in its only experimental tests (using *Drosophila*⁶³, *Kalanchoe*

flowers⁶⁴ and sparrows⁶⁵) makes it worthwhile to test directly its underlying assumption that the circadian mechanism, being a simple clock, relaxation oscillator, or quickly-recovering limit-cycle mechanism, essentially pops from one point to another on its cycle when reset.

Checking this assumption would be unnecessary if the circadian oscillator were a simple clock or relaxation oscillator, which have no accessible states other than the usual cycle. The fact that rephasing curves are smooth and change abruptly at a critical stimulus duration from mean slope 1 to mean slope 0 both excludes those two models and shows that the POPP rule requires amendment for application to limit-cycle mechanisms: for if all stimuli leave the oscillator very nearly on its limit cycle, then a long stimulus, evoking type 0 resetting, can be viewed as an uninterrupted sequence of shorter stimuli, but under POPP rules, any sequence of type 1 stimuli still gives type 1 resetting. Perhaps the POPP rule must be restricted to 'strong' stimuli or to stimuli that are 'not too close together'. In measuring how strong is 'strong' or how close is 'too close', we determine how quickly the circadian oscillator recovers to its limit cycle after a disturbance. Such an experimental test of the POPP rule in *Kalanchoe* discovered no systematic deviation from its predictions for stimuli at least 2 h apart⁶⁴, though the data fit as well to a limit-cycle model with recovery time in the order of a day^{56,57}.

But in *Drosophila*, no evidence of recovery was found. In 600 experiments, a once-reset rhythm was subjected to a strong second stimulus at various times to measure the rephasing curve of a previously reset oscillator: how quickly does it return to normal, leaving only a phase shift equal to that observed in the overt behavioural rhythm? The normal phase-dependence of the phase response was found to be attenuated in proportion to the nearness of the first stimulus to the singularity, and there was no sign of return to normal during the 48 h examined by a second pulse, for such stimuli, any interval is 'too close'. No such attenuation was conspicuous following stimuli far from the singularity, for such stimuli no interval is 'too close'^{56,67}.

So the fly's clock does not resemble a quickly recovering limit cycle mechanism, either. What it does resemble is a simple harmonic oscillator—for example a pendulum—in which disruptive stimuli result in permanent, co-ordinated shifts of both phase and amplitude without effect on frequency. In fact, a mathematical metaphor of this sort fits the data from over 1,000 diverse one-pulse and two-pulse rephasing experiments on the circadian rhythm of eclosion of *Drosophila* to within almost the reproducibility of measurements (ref. 66 and A.T.W. in preparation). The theory of isochrons and phase resetting which originally predicted the singular stimulus^{50,60} cannot be rigorously applied to such mechanisms.

Organism as a clockshop

What can such a grotesque coincidence possibly signify? The thought that most tantalises me is that the individual fly's behavioural rhythm may be determined by superposition of at least two independent circadian oscillations, each possibly arising from a simple clock, relaxation oscillator, or limit cycle mechanism. (Care was taken⁶⁸ to exclude the possibility^{56,57,64,66,70} that attenuated rhythmicity arose as an artefact of phase incoherence among individually coherent pupae of the sampled populations.)

From such indications as the overt desynchronisation of circadian rhythms among the organs and cells of higher plants⁶, the splitting of activity rhythms in flies⁷¹, bees⁸, birds⁷³, rodents⁷⁴ and primates⁷⁵, some workers have speculated that the organisms more resemble a clockshop

than a clock^{70,73-80}. Populations of weakly interacting idealised oscillators have been shown to exhibit mutual synchronisation, splitting, and more complex quasi-rhythmic idiosyncrasies resembling those observed in large multicellular organisms^{44,81}. By an adjustment of the phase relation between their mutual influence and sensitivity rhythms, they can just as easily repel each other to remain uniformly distributed around the cycle⁴⁴. Thus overt arrhythmicity in some species is not incompatible with the notion that cellular metabolism is typically organised in 24-h patterns.

Since direct control of circadian rhythms by sensitive extra-retinal photoreception is quite common, even among vertebrates²⁻⁶, there might be little need even for weak interactions among independent cellular oscillators, all cells being synchronised in parallel directly by the external light/dark cycle. Only when completely coherent does such a population exhibit the resetting characteristics typical of the individual oscillator mechanism. This may be the situation in entrainment experiments, and the deepest reason for success of the POPP model's predictions for entrainment, despite the failure of its prediction for general two-pulse interaction.

Even though a simple clock, for example, cannot show type 0 rephasing or a singularity, the summed outputs of a population of simple clocks can if its independent members are not quite synchronous when the stimulus starts. A properly timed stimulus of the right duration greatly increases the incoherence of such a population, resulting in attenuation of the collective rhythm and correspondingly attenuated rephasing curves. Both persist as though the 'amplitude', 'intensity' or 'vigour' of circadian oscillation had been reset without affecting its period, because without interactions and without much dispersion of cellular periods, the variance of cellular phases would not change. This variance of cellular phases may be the 'second and uncontrolled experimental variable' suggested above.

These matters are elaborated in more detail (A.T.W. in preparation) elsewhere: the point I wish to make for present purposes is that the unclocklike peculiarities encountered in the most thoroughly studied circadian rhythms might be accounted for in three different ways.

(1) Without invoking a multiplicity of independent oscillators, by finding a plausible mechanism with amplitude as labile as phase, and frequency nearly independent of amplitude. Predator-prey kinetics on a chemical level has this character⁸². Goodwin^{15,83} proposed a mechanism based on control of nuclear DNA-dependent RNA synthesis which could oscillate with a period of many hours in the required orbitally stable way.

(2) By supposing that whole-organism behaviour is governed by the superposition of two identical and independent smooth limit-cycle oscillations of 24-h period, perhaps one on each side of the brain^{71,72}. When their phases differ greatly, for example after both have independently recovered to random phases from near the singularity, the 24-h component of their combined outputs would be quite weak. This idea could be tested by one-sided surgical "clockectomy".

(3) By taking seriously the evidence noted above that circadian rhythmicity is an autonomous cellular process. If the cells are assumed not to interact much (as in a clockshop), then whether they are simple clocks, relaxation oscillators, or limit cycle mechanisms, the qualitative peculiarities of rephasing behaviour follow, thus harmonising phylogenetically widespread rephasing behaviour with the apparent diversity of evolutionary origins and physiological mechanisms of circadian rhythmicity.

On this alternative, the damping rate of free-running rhythms measures the range of individual cell periods and the 'clockshop' conjecture is implausible to the extent that this range is implausibly small. Persistence of rhythmicity in

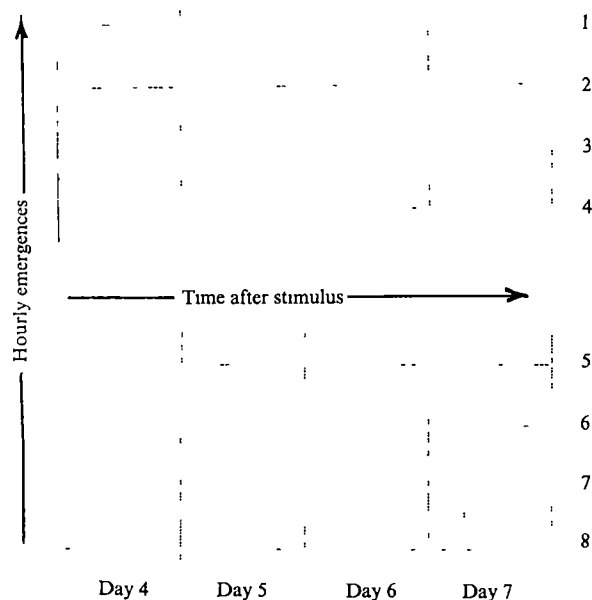


Fig 4 The eclosion rhythm of 8 populations of *Drosophila* is recorded hourly through days 4–8 after stimulus time. Each mark in the histogram represents 1 fly. Populations 1–4 received about 5,000 erg cm⁻² of blue light near subjective midnight. Their rhythmicity is attenuated relative to populations exposed at any other combination of intensity and phase, in all cases, for example histograms 5–8, the normal rhythm is merely phase-shifted (in Figs 2 and 3, peaks like the 15 shown here are each represented by a single dot).

Drosophila for at least 1 d without indication of damping^{28, 70} suggests a dispersion of periods smaller than 2%. In *Gonyaulax* cell suspensions, rhythmicity of cell division persists unattenuated for at least 10 days⁴¹. Assuming no mutually synchronising interactions, this also suggests that cell clocks differ in period by less than about 2%. Chemical interactions were tested for by mixing differently phased cell suspensions without revealing any tendency to synchronisation^{45–87}. The corresponding test in multicellular animals or plants would be parabiosis of individuals reared on different light cycles, or parabiosis of a rhythmic individual with one rendered arrhythmic by the singular stimulus. Discovery of strong synchronising interactions would eliminate the 'clockshop' conjecture⁸⁸ (Of course electrical interactions among nerve cells would escape detection by these means).

Finally, this model predicts that an organism in the low-amplitude condition should exhibit a conspicuous scattering of cell phases. No such dispersion of cellular clocks states is predicted under the orbitally stable oscillator model (1). Since some cells exhibit circadian rhythms of nuclear volume, chloroplast shapes and so on⁶, this might afford a critical test of the 'clockshop' conjecture.

Either way, there remains the Darwinian question: given labile amplitude, how does it fit into the organism's life style? Has it a selective advantage or is it an 'accidental' selectively neutral by-product of something more important? My guess is that the clockshop hypothesis is correct, and that 'amplitude' lability is a laboratory artefact, the cellular incoherence necessary for its appearance being rare under field conditions of entrainment to natural photoperiods. The possibility should, however, be investigated that this 'unclocklike' feature of circadian organisation is hinting to us of some adaptive role other than the 'time' and 'phase'-oriented purposes considered up to now.

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articles

Mesozoic and Cainozoic opening of the Labrador Sea, the North Atlantic and the Bay of Biscay

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A reinterpretation of magnetic anomaly and basement trends suggests that the Labrador Sea opened in two stages. During a first phase, most likely in the late Jurassic–early Cretaceous, the opening was coupled, by way of a triple junction off Spain, to spreading in the central Atlantic and Bay of Biscay. A second phase of seafloor spreading in the Labrador Sea occurred between 60 and 47 Myr ago and was connected through a triple junction south of Greenland to spreading in the northern and central Atlantic.

THE Labrador Sea is thought to have formed as the result of the rifting apart of Labrador and Greenland and the formation and spreading of the sea floor in between^{1–11}. The correlation of magnetic and basement trends presented here differs from that of previous workers^{4,7,11,12} and points to an alternative geometry and spreading history for the Labrador Sea and the North Atlantic Ocean.

Magnetic trends

The residual magnetic field over the north-western Atlantic Ocean and the Labrador Sea (Fig. 1) is derived from maps in refs 4, 11, 12 and 13. Although there are inconsistencies, the overall trends and characteristics of the anomaly sequences match very well.

Over the Labrador Shelf, Precambrian rocks, covered by a wedge of seaward-thickening sediments, produce a pattern of chaotic high frequency anomalies. The zone beyond the shelf edge, which is 170 km wide and underlies the Continental Slope and Rise, is interpreted as foundered continental crust¹³. The magnetic field over this zone is characterised by small amplitude, large wavelength anomalies that run parallel to the margin. The approximate seaward limit of this zone (Fig. 1) is interpreted as the true boundary between continent and ocean.

In the Labrador deep sea, there are two sequences of oceanic anomalies with a regular and parallel pattern. Sequence I comprises all anomalies that run NW–SE, in the northern and in the southern parts of Fig. 1, although they are partially separated by a central zone of attenuated anomalies. The attenuated anomalies outline a basement high (the Cartwright Arch), which is the offshore extension of the Grenville Province¹³. Sequence II, labelled 19–24 in Fig. 1, runs almost due north from the Charlie Gibbs Fracture Zone up to about 56°30'N. There it bends westwards through approximately 80°,

and continues along this trend for about 200 km. Here the sequence changes direction again and at the same time the anomalies converge, they finally wedge out at approximately 57°N 50°30'W.

The termination of sequence II provides the key to the new correlation. The pattern is more clearly illustrated in Fig. 3a of Vogt and Avery¹¹, where the anomalies are presented with a contour interval of 50 gamma. The new correlation differs from earlier correlations^{4,7,11,12}, which, despite the pinching out near 57°N, continue sequence II into the northern part of sequence I.

The age of sequence II has been defined accurately using the time scale of Heirtzler *et al.*¹⁴. A match was obtained with anomaly sequence 19–24 which indicates that spreading occurred 47 to 60 Myr ago^{4,12}. The age of sequence I has not been accurately determined and various spreading periods have been postulated: 65–80 Myr (ref. 3), 60–80 or 82 Myr (refs 4 and 12), 60–76 Myr (ref. 5) and 38–52 Myr (ref. 7). The estimates (with one exception⁷) focus on a period, immediately prior to the generation of sequence II, in the late Cretaceous and early Tertiary. Many authors have considered an even earlier (perhaps epicontinental, perhaps rifted) Labrador Sea, in existence before the generation of anomaly sequence I (refs 2, 4, 5, 10, 11, 15, 16).

Basement trends

The basement morphology of the Labrador Sea region south-west of Greenland parallels the magnetic anomalies. In Fig. 1, I have indicated the culminations of basement as defined in refs 1, 4, 5, 9, 12, 13 and 15. The authors interpreted these basement highs as expressions of fossil mid-ocean ridges or former centres of seafloor spreading. Laughton considered the east–west trending basement structures at about 57°N to be continuous with north-westerly trending basement structures in the northern Labrador Sea. Vogt and Avery¹¹ accept this interpretation and propose the name Ran Ridge for this fossil mid-ocean ridge.

The correlation of basement trends is similar to that of the magnetic trends. Thus, the NW–SE trend of the mid-Labrador basement highs, parallel to magnetic sequence I, is probably continuous from the northern Labrador Sea to the Charlie Gibbs Fracture Zone. Also the east–west trending ridge, parallel to magnetic anomaly sequence II, probably terminates at about 51°W. In Fig. 1, the magnetic lineations associated with the

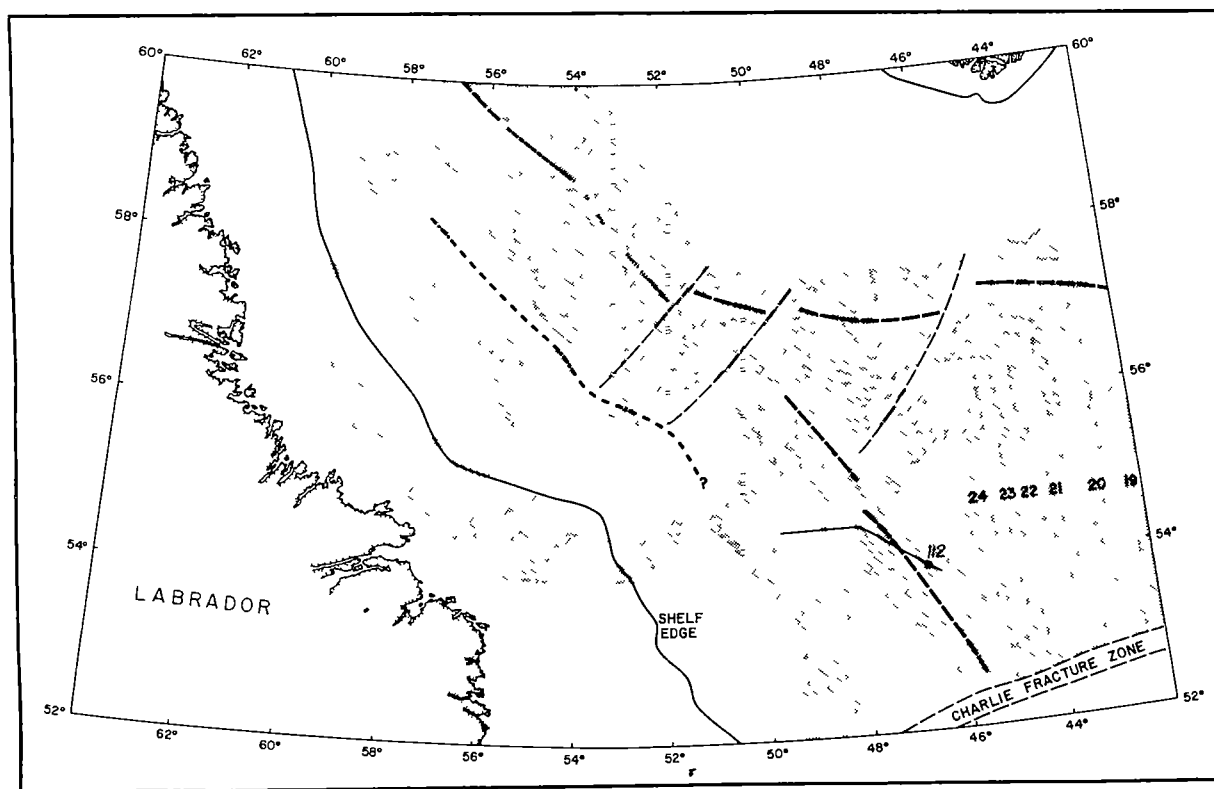


Fig 1 Observed (— — —) and inferred (---) positions of basement highs superimposed on the distribution of positive (shaded) and negative magnetic anomalies in the Labrador Sea (after refs 4, 5, 9, 11 and 13) — — — —, The inferred continental-ocean boundary. Correlation of anomalies with the time scale of Heirtzler *et al*¹⁴ is shown for the sequence 19–24. DSDP site 112 is marked, as well as the position of a CSS Hudson reflection profile (Fig 5) through the site

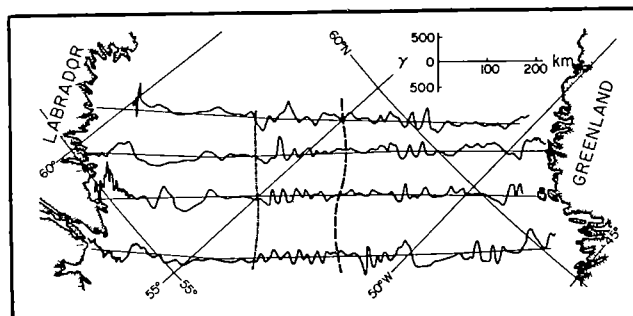
eastern half of the NE-SW spreading are only partly shown. Information from south of Greenland is too sparse and has not been included on the map. Farther north, however, aeromagnetic surveys^{7, 17} indicate a mirror image of the western Labrador magnetic anomalies on the Greenland side (Fig 2). A belt of relatively high frequency anomalies can be distinguished on both sides of a central low amplitude sequence. The centre of spreading (Fig 2) coincides with the position of the fossil mid-ocean ridge (Fig 1).

Labrador Sea and North Atlantic

Figure 3 combines the information for the Labrador Sea with the magnetic anomaly pattern and major structures of the North Atlantic Ocean. Sequence II (anomalies 19–24) is very well defined and can be easily correlated, particularly north of the Charlie Gibbs Fracture Zone (CGFZ)^{9, 11}, where it is undisturbed by fracture zones. In contrast, the correlation of anomalies and comparison with the magnetic time scale are often ambiguous for sequence 5–18 (9–46 Myr BP), especially south of the CGFZ. The various isochrons, both north and south of the CGFZ, each identified with their age in millions of years, were taken from Pitman and Talwani¹⁸. The anomaly pattern in the Bay of Biscay and the area immediately to the west of the Bay is taken from Williams¹⁹. Vogt and Avery¹¹ indicate two basement ridges, West Thulean Rise and East Thulean Rise, about equidistant on either side of the Mid-

Atlantic Ridge, south of the CGFZ. These ridges are significant in the reconstruction of the North Atlantic, as will be shown. In the eastern Atlantic, north of the CGFZ, the magnetic anomalies run in a south-easterly direction^{4, 11, 12} (oblique to the 19–24 sequence to the west and parallel to the East Thulean Rise). Across the CGFZ, these anomalies do not match closely the anomalies shown by Williams¹⁹ west of the Bay of Biscay. Certainly the 81-Myr isochron through this area¹⁸ is doubtful.

Fig 2 Four aeromagnetic profiles across the northern Labrador Sea (after Hood and Bower⁷) with estimated position of centre of symmetry (---) and inferred continent-ocean boundary (---). Amplitude scale in gamma.



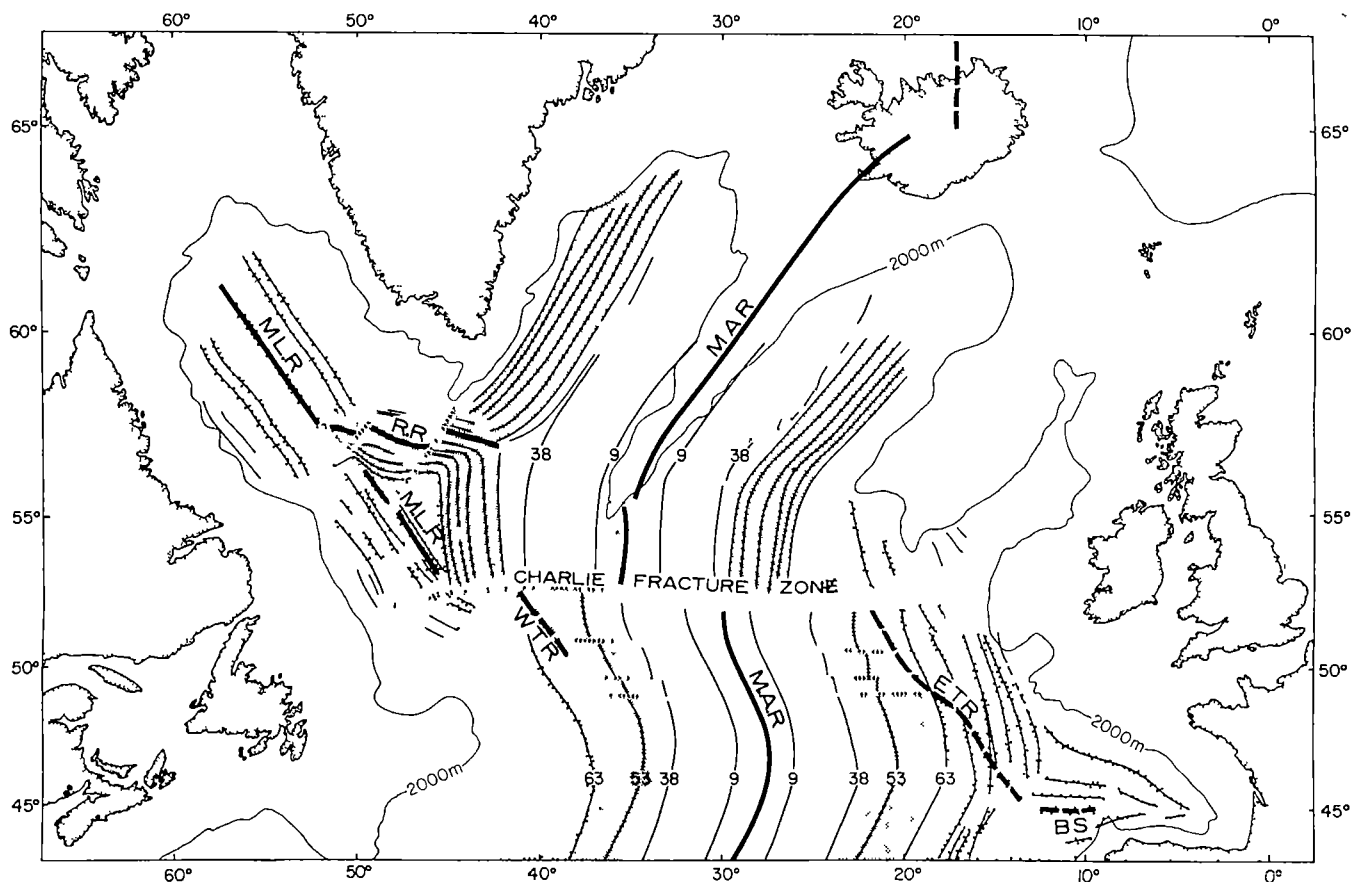


Fig. 3 Position of spreading centres (— — —), major faults (— — —), magnetic anomalies and isochrons (labelled) in the North Atlantic Ocean, modified after refs 9, 11, 18 and 19. Magnetic anomaly sequence 19–24 (47–60 Myr BP) is densely shaded. Seafloor area generated during the Mesozoic is approximated with open shading. MAR, Mid-Atlantic Ridge, MLR, Mid-Labrador Ridge, RR, Ran Ridge, WTR, West Thulean Rise, ETR, East Thulean Rise, BS, Biscay Seamounts.

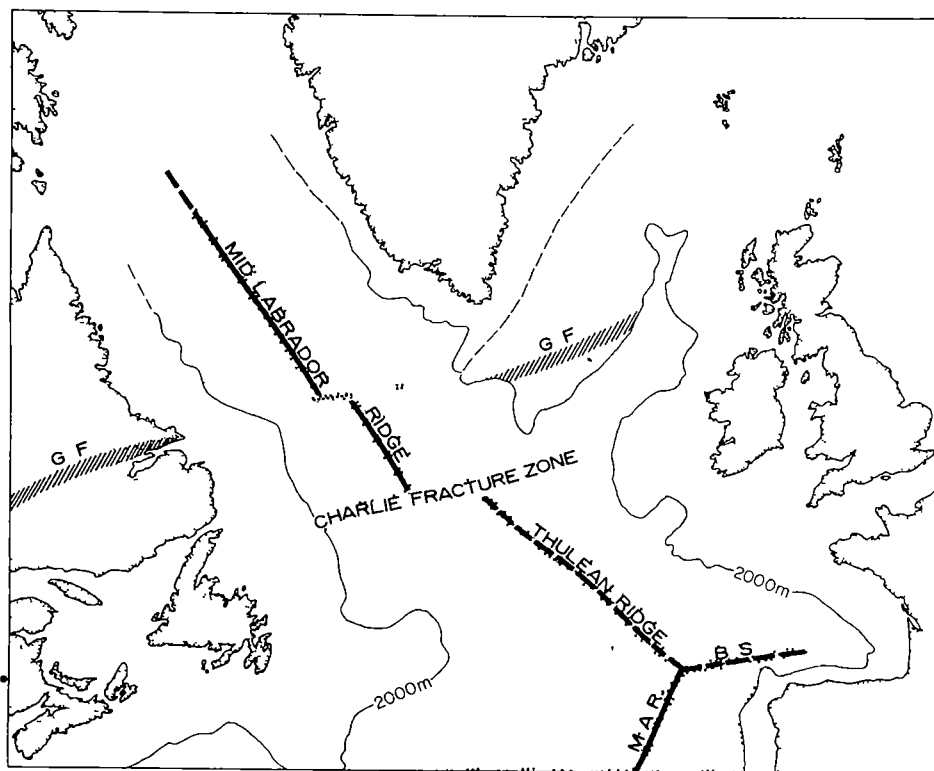


Fig. 4 The North Atlantic Ocean at the end of the Mesozoic. Seafloor area generated during the Mesozoic (phase I) is approximated with shading. The reconstruction is produced by removing the seafloor generated in the past 60 Myr (phase II and younger) and closing the gap. Note that the transform fault north of the Charlie Gibbs Fracture Zone approximates the position of the Ran Ridge. MAR, Mid-Atlantic Ridge, BS, Biscay Seamounts, GF, Grenville Front.

and is thus not included in Fig 3 The 81-Myr isochron off the Grand Banks of Newfoundland is also omitted, for similar reasons

I would like to retain the old name Mid-Labrador Ridge for the spreading centre, about mid-way between Labrador and Greenland, and to restrict the name Ran Ridge¹¹ to the east-west trending ridge, centred on anomaly 19

The following reconstruction is proposed for the Labrador Sea Phase I—the Labrador Sea opens between Greenland and Labrador with spreading centred on the Mid-Labrador Ridge Phase II (60–47 Myr BP)—seafloor spreading is centred on the Ran Ridge and coupled through a triple junction to spreading on the Mid-Atlantic Ridge

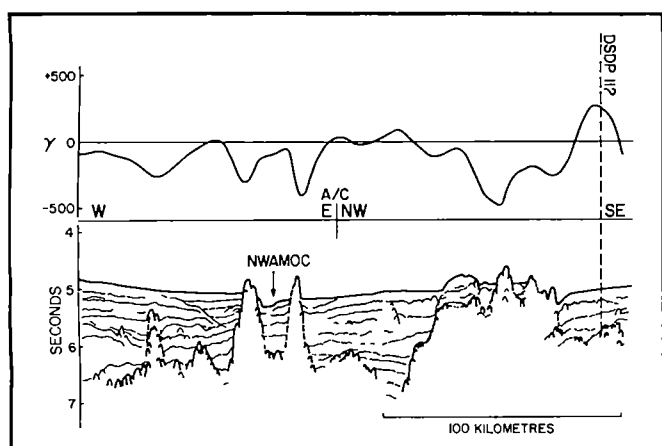


Fig 5 Line tracing of 1,000 inch³ air gun reflection profile and associated magnetic anomalies in the Labrador Sea (CSS Hudson, cruise 72–025). Vertical scale for the reflection profile is in seconds two-way travel time. Vertical exaggeration 33:1 at water velocity. Vertical scale for the magnetic profile is in gamma. NWAMOC, North West Atlantic Mid-Ocean Channel. DSDP 112, Deep Sea Drilling Project site 112. For the position of the line see Fig 1. Note the coincidence of the positions of negative magnetic anomalies and positive basement relief.

It is suggested that the spreading activity in the Labrador Sea during phase I was coupled to spreading in the Bay of Biscay^{15,20}. About 60 Myr ago the East and West Thulean Rises were one ridge system, as can be demonstrated by reversing the spreading in the Atlantic since that time (Fig 4). Williams¹⁹ suggested a triple junction that tied the largely north-south directed opening of the Bay of Biscay to east-west directed seafloor spreading in the eastern Atlantic Ocean during the Mesozoic. Thus, spreading was centred on the Mid-Labrador-Thulean ridge system, Biscay Seamounts, and Mid-Atlantic Ridge. The reconstruction (Fig 4) suggests that during phase I the Ran Ridge was a transform fault that trailed the Grenville Front¹³.

Age of spreading

The age of seafloor spreading during phase I is not firmly established but it certainly predates spreading during phase II. The shift of 25° from phase I (NE–SW spreading) to phase II (east–west spreading) (Fig 1) constitutes a major change in the orientation of plates that most likely represents a hiatus in the spreading of the Labrador Sea.

Support for a late Cretaceous–early Tertiary age for phase I comes from Deep Sea Drilling Project (DSDP) site 112 (ref 21). Basement samples cored at the site consisted of weathered basalt that could not be dated. A minimum age of 65 Myr was inferred for the basement from the age of the oldest sediments cored above the basalt. A seismic profile through the site taken by CSS Hudson (Fig 5) indicates that this inference is

misleading. Eighty kilometres to the north-west the thickness of the sediments above the basement is twice the thickness found at site 112, which suggests a minimum age of the basement of 130 Myr. If a contemporaneous opening of the Labrador Sea and the Bay of Biscay is accepted then the identification of magnetic anomalies 31–32 in the Bay¹⁹ supports a late Cretaceous age for episode I. An earlier interpretation of the same anomaly pattern²², however, suggests a late Cretaceous age for the Bay of Biscay.

There are other arguments in support of an earlier, possibly Jurassic phase of opening of both the Labrador Sea and the Bay of Biscay. On the basis of geological and geophysical evidence from both Spain and France (stable Europe), the Bay of Biscay is considered to have been formed between the Triassic and late Cretaceous²³. Results from DSDP sites 118 and 119 in the Bay of Biscay suggested to Laughton¹⁷ that the oldest sediments below the deepest dated horizon in the Bay (Palaeocene) may be as old as early Cretaceous. Palaeomagnetic results suggest an opening of the Bay of Biscay some time between the Triassic and late Cretaceous²⁴. Larson and Pitman²⁵ elaborated on the interpretation of Williams and McKenzie²² and suggested that the Biscay anomalies may be part of the Keathley sequence and thus that the Bay opened between 150 and 110 Myr BP. Correlation of the Labrador Sea and Keathley anomalies is indeed at least as good (or as bad) as the correlations between the Labrador anomalies and the Cretaceous magnetic timescale. The position of the Keathley anomalies in the central Atlantic^{18,26} would then identify the position of the third or southern Middle Mesozoic spreading arm, radiating from the Williams¹⁹ triple junction.

As mentioned, several authors favour partial opening of the Labrador Sea during Jurassic time, although no one has suggested a Jurassic age for the northern Labrador magnetic anomalies. McMillan^{10,16} argued for a Mesozoic initiation of the Labrador Sea to explain the occurrence of Jurassic carbonates collected from the Labrador continental slope.

Further support for late Jurassic–early Cretaceous rifting of the Labrador Sea comes from a series of dikes that run parallel to the coast in south-west Greenland²⁷, these have been dated as late Jurassic (138 Myr). Dike intrusion may, however, have predated actual spreading by several million years.

From the available evidence, I am inclined to accept a late Jurassic age as the most plausible for the opening of the Labrador Sea and the Bay of Biscay. A more definitive answer to the problem will have to come from more closely spaced magnetic surveys and from dated oceanic basalts in crucial areas.

I propose that (1) the Labrador Sea was formed during two phases of seafloor spreading, one in the late Jurassic–early Cretaceous and one in the early Tertiary, and (2) the Labrador Sea opening in the Mesozoic was coupled with seafloor spreading in the North Atlantic Ocean and the Bay of Biscay.

As an alternative to earlier interpretations^{4,5,12}, this model simplifies the geometry of the Atlantic evolution. A Jurassic age, suggested for the early phase of opening, is in better agreement with geological observations on the Labrador Sea margin and the Bay of Biscay. It also suggests that Mesozoic seafloor spreading in the Atlantic Ocean was not restricted to the area south of the Azores–Gibraltar Fracture Zone¹⁸.

There are several areas which have to be examined if the model is to be refined—notably, between the East Thulean Rise and Europe, between the West Thulean Rise and the Grand Banks, and between Greenland and the Ran Ridge. These areas are all near continental margins and are probably structurally complex.

Drs L. H. King and R. K. H. Falconer reviewed the manuscript.

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Nucleic acid-mutagen interactions: crystal structure of adenylyl-3',5'-uridine plus 9-aminoacridine

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Adenylyl-3',5'-uridine and 9-aminoacridine cocrystallise in a heavily hydrated monoclinic lattice. The adenine residues are hydrogen bonded with uracil residues and the base pairs are stacked parallel to each other in the crystal with 9-aminoacridine sandwiched directly between them.

In 1961 Lerman¹ suggested that planar aromatic aminoacridine molecules can be inserted between the base pairs in DNA. This mode of molecular interaction has been widely accepted and many interactions between nucleic acids and planar molecules have been explained in terms of an intercalation model. A number of varied molecules including carcinogens and mutagens have a mode of action which is believed to be intimately associated with the intercalation mechanism. In spite of a great deal of chemical and biological work, however, the detailed structure of intercalation complexes has not been determined. In part, this reflects the fact that it is difficult to obtain structural information at atomic resolution from X-ray diffraction studies of polymeric nucleic acids combined with intercalating agents. In view of the large number of important biological phenomena associated with intercalation, it is desirable to understand the detailed interactions of planar intercalating molecules with hydrogen bonded base pairs. Here we report the crystal structure of complex containing the dinucleoside phosphate adenylyl-3',5'-uridine (ApU) and the mutagen 9-aminoacridine. In the heavily hydrated crystal lattice the adenine and the uracil residues are hydrogen bonded while the 9-aminoacridine molecules are sandwiched between the base pairs and stacked parallel to them.

Experimental details and solution of structure

A solution of 5 mg of ApU (Sigma) in 0.25 ml of a 200 mM sodium cacodylate buffer at pH 6.6 was prepared. A 4.16 mg sample of 9-aminoacridine hydrochloride was dissolved in this solution, followed by the addition of 0.1 ml of isopropanol. The vial was sealed, and crystals suitable for diffraction analysis appeared within a week. These crystals were unstable in the

absence of the mother liquor and had to be mounted in a sealed capillary. The lattice constants were determined and intensity data were collected on a Syntex PI diffractometer. The crystal data are $a=13.261 \text{ \AA}$, $b=26.677 \text{ \AA}$, $c=6.922 \text{ \AA}$, $\beta=95.08^\circ$, space group $P2_1$. Intensity data were collected for 3,553 Friedel pairs using nickel filtered $\text{Cu K}\alpha$ radiation. 2,874 reflections were observed on the basis of a 2σ significance criterion. Lorentz and polarisation corrections were applied to the intensity data, but no absorption correction has been calculated.

The standard method for solving crystal structures of dinucleoside phosphates has been to locate the phosphorus atom by means of resolution difference techniques²⁻⁴. Many attempts to apply this technique to this structure were unsuccessful because the phosphorus-phosphorus vector was hidden under a large peak on the Harker section. We were therefore forced to locate the phosphorus atom by the use of the anomalous dispersion data available from the phosphorus which has 0.43 anomalous electrons. Once the phosphorus was located, the rest of the structure was readily obtained in a series of seven Fourier cycles and the water structure was determined in a series of difference Fourier calculations. The crystal structure has been refined using least squares and anisotropic thermal parameters to a conventional R factor of 7%. The disorder in the water structure has made it impossible to locate hydrogen atoms.

Description of the structure

The molecular structure and numbering for ApU and 9-aminoacridine are shown in Fig. 1. Figure 2 shows a view of the complex perpendicular to the plane of the adenine-uracil base pair and the 9-aminoacridine molecule. In Fig. 2 the monoclinic b axis is in a horizontal orientation and the twofold screw relationship between successive molecules is clearly shown in the diagram. The thickness of the unit cell perpendicular to the plane in Fig. 2 is 6.9 \AA which is twice the thickness of the planar aromatic rings. It can be seen that the adenine-uracil residues are hydrogen bonded in pairs using the nitrogen 6 of adenine bonding to oxygen 4 of uracil (hydrogen bond length $=2.99 \text{ \AA}$) and nitrogen 3 of uracil bonding to nitrogen 7 of adenine (2.84 \AA). This hydrogen bonding arrangement, first observed by Hoogsteen⁵, has been seen in a large

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number of purine-pyrimidine complexes⁶ The hydrogen bonded base pair is surrounded on both sides by 9-aminoacridine molecules as shown in Figs 2-4 In addition to this, the adenine residues are hydrogen bonded to the ribose hydroxyl groups of an adjoining uridine where the interactions are between the N6 amino group of adenine and O3' of the ribose (2.95 Å) and between N1 of adenine and O2' of the same uridine ribose (2.77 Å) Although the hydrogen bonding to uracil involving the adenine imidazole N7 nitrogen has been seen in many complexes, the hydrogen bonding between adenine and ribose in this specific manner has not been seen previously

The projection in Fig 2 shows an unusual circular cylindrical channel, the walls of which are composed of four different molecules of ApU and three molecules of 9-aminoacridine The entire structure is built out of clusters of these cylindrical complexes, one complete unit of which is shown in Fig 2 The interior of the cylinder is heavily hydrated containing 15 water molecules which are disordered among 21 loci in the asymmetric unit Since these hydrated channels go through the entire crystal, it is likely that this gives rise to the instability of the crystal on drying

Figure 3 shows a view of one segment of the wall of the cylindrical channel viewed perpendicular to the direction shown in Fig 2 Here the insertion of the planar 9-aminoacridine molecules is shown between the adenine-uracil hydrogen bonded pairs The adenine-uracil pairs are nearly coplanar, but have a slight propeller twist of 4° between them

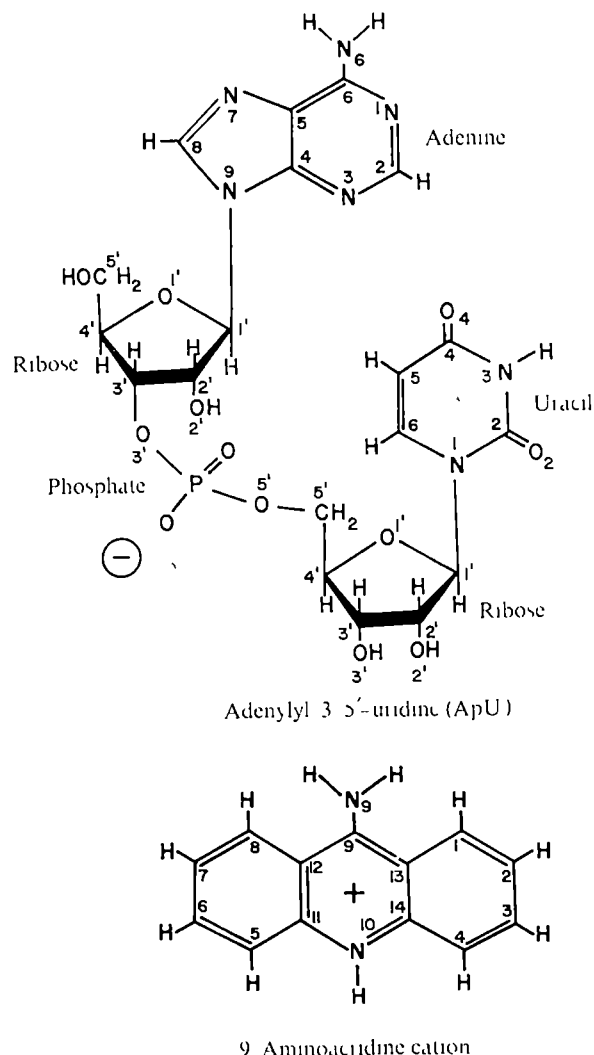


Fig. 1 The chemical formulae and molecular numbering system for adenylyl-3',5'-uridine and 9-aminoacridine

Nucleoside conformations are unusual

The perturbation of the dinucleoside phosphate structure by the mutagen results in some unusual features for the nucleosides The adenosine ribose has a C2' endo conformation with a glycosidic torsion angle of 77° which is in a region between the syn and anti conformations^{7,8} The glycosidic torsion angle is not unusual for nucleosides which have a C2' endo ribose, however, this is the first time that a C2' endo conformation has been seen in a dinucleoside ribophosphate The ribose of the uridine nucleoside is in the more familiar C3' endo conformation, however, the glycosidic torsion angle is again

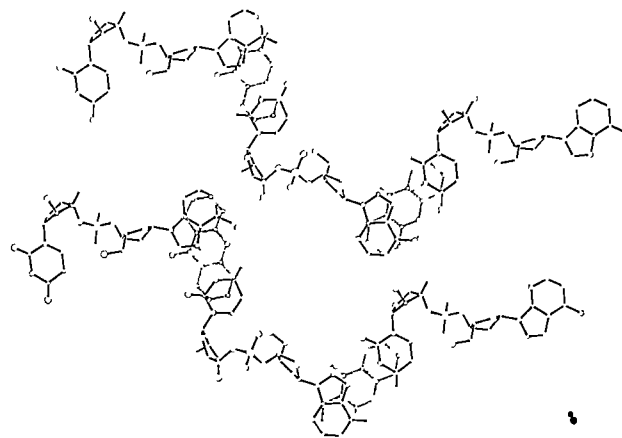


Fig. 2 A view of the structure perpendicular to the planes of the bases and 9-aminoacridine The 9-aminoacridine does not have darkened bonds The dashed lines indicate the hydrogen bonding interactions of adenine The *b* axis is horizontal, and the cylindrical water channel is seen here

rather unusual having a value of 71° which has not been reported previously for the 3' endo sugars As can be seen in Fig 3, the bases are approximately parallel to the ribose O1'-C4' bonds rather than O1'-C1' as is usually the case^{8,9} It is interesting that the puckering of the adenosine ribose ring and the values of both glycosidic torsion angles are characteristic features of deoxyribonucleotides rather than ribonucleotides¹⁰

The backbone connecting the two nucleosides is in a helix-reversing A1 conformation⁹ which was first seen in the crystal structure of UpA² All the bond angles and distances in the dinucleoside phosphate are in the normal range, the only unusual features of the structure associated with the perturbation caused by the acridine are the unusual glycosidic torsion angles and the conformation of the adenosine ribose ring In contrast to this the torsion angles of the backbone are comparable with those which have been observed in all previous dinucleoside phosphate structures^{2-4,11} It is interesting to ask what would happen if one inserted the adenosine and uridine nucleosides observed here into a double helical structure such as those found for the sodium salts of ApU³ and GpC⁴ The result would be a structure with an increased distance between the adjacent bases along the chain The normal 3.4 Å stacking distance would be approximately doubled, allowing the acridine to be intercalated between the base pair

A view of the hydrophobic intercalated column containing the acridines and the base pair is shown in Fig 4 where we are viewing the column at an angle which allows us to visualise both the base pair and the acridine atoms The unusual glycosidic torsion angles can be seen in this projection as well as in the projection shown in Fig 3

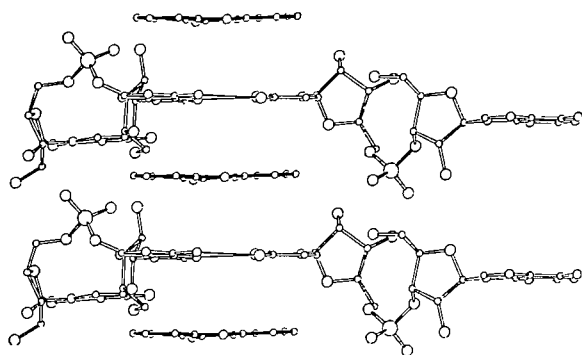


Fig 3 A view of the intercalative structure perpendicular to the view in Fig 2. The bases and 9-aminoacridine are viewed end on. The acridine molecules have darkened bonds.

Stacking interactions

Water molecules are excluded from the hydrophobic column shown in Figs 3 and 4, and this undoubtedly contributes to its stability. The stacking interactions are very great in this complex and these no doubt provide the driving force for forming the complex. The water excluded from these areas is found in the elongated channels. The detailed nature of the water structure will be described elsewhere.

Another feature of the present structure is the fact that electrostatic interactions probably play only a small role in stabilising the structure. The acridine molecule is charged and, as shown by Talacki *et al.*¹², part of the positive formal charge resides on the amino group and part is on N10. These atoms are both more than 6 Å away from the nearest phosphorous atom. Because of this considerable distance electrostatic interactions are likely to be far less important in stabilising the structure than the considerable stacking interactions. It is interesting that the acridine C9-N9 bond is antiparallel to and almost overlapping the adenine C8-N7 bond. This and other overlaps seen in Fig 2 suggest that the fine details of the stacking may be stabilised by small local electrostatic interactions.

The detailed nature of the interaction of the 9-aminoacridine with the base pair is of interest. Figure 2 shows that each of the two outer six-membered rings of the acridine is largely overlaid by a purine or a pyrimidine. The centre ring is largely found in the region occupied by the hydrogen bonding interactions. The relationship of the stacking interactions seen with this hydrogen bonding to the interactions which might be found in a double helix involving the normal Watson-Crick hydrogen bonding is not entirely clear. One possibility, however, is that the interaction of the 9-aminoacridine with either the uracil or the adenine might be preserved in a double helix. If the uracil-acridine interaction were conserved with a normal Watson-Crick base pair, heavy overlap with the adenine would result. Alternatively, if the overlap with the adenine residue were maintained, there would be no overlap between acridine and the uracil. However, in this latter position the 9-amino group of the cationic mutagen would be in a position to form strong hydrogen bonds with the anionic phosphate group. It is interesting that two different binding modes have been described for intercalating agents¹³.

Features of this mutagen-dinucleoside phosphate complex might have relevance in other structures. It is known that planar intercalative molecules can combine with transfer RNA¹⁴. There are two hydrogen bonding interactions in yeast phenylalanine tRNA which involve uracil derivatives hydrogen bonding to imidazole N7 of adenine^{15,16}. It is conceivable that the interaction of planar intercalating molecules may interact at these sites in a manner similar to that seen in this structure. Another interesting feature of the present

structure is the unusual hydrogen bonding found between the ribose residue and the adenine ring. This type of interaction might be specific for adenine and it might be associated with some of the constant adenine residues which are found in tRNA where they may hydrogen bond to ribose residues.

This is the first molecular structure analysis carried out at atomic resolution which clearly indicates the mode of intercalation of a mutagen between an adenine-uracil base pair. The structure has some unusual features, some of which are suggestive of the manner in which planar molecules may be intercalated into double helical structures or into other more complex nucleic acid structures such as tRNA or ribosomes. This structure illustrates one stable mode of interaction between a mutagen and a base pair. Aspects of this structure may be of help in providing a detailed understanding of the mode of action of mutagens in biological systems.

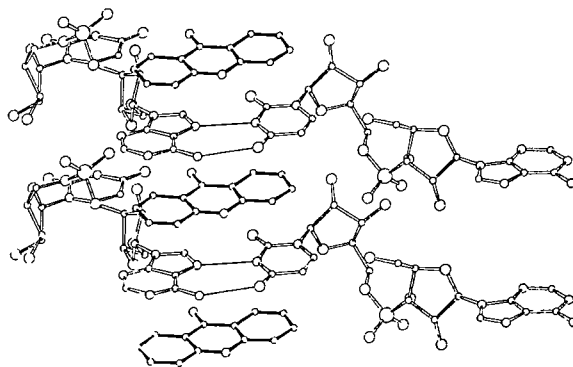


Fig 4 A view of the intercalative stacking interaction at an oblique angle. The nature of the vertical hydrophobic column of alternating base pairs and 9-aminoacridine is seen here.

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Note added in proof: We were recently sent a preprint of a paper by C. C. Tsai, S. C. Jain and H. M. Sobell describing the crystal structure of an ethidium-dinucleoside phosphate complex in which ethidium is intercalated in a fragment of RNA double helix¹⁷. The nucleosides show some of the same perturbations described here. We thank Dr Sobell for providing us with this information.

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letters to nature

Jupiter's main magnetic field measured by Pioneer 11

THE first *in situ* studies of the magnetosphere of Jupiter were conducted in November and December, 1973, by the spacecraft Pioneer 10. An analysis of the helium magnetometer data¹ indicated that the planetary magnetic field was well represented by an offset tilted dipole (OTD) at distances less than $10R_J$ (R_J =radius of Jupiter=71,372 km). But the movement (4.0 gauss R_J^{-3}) and tilt (10.6° at $\lambda_{III}=$

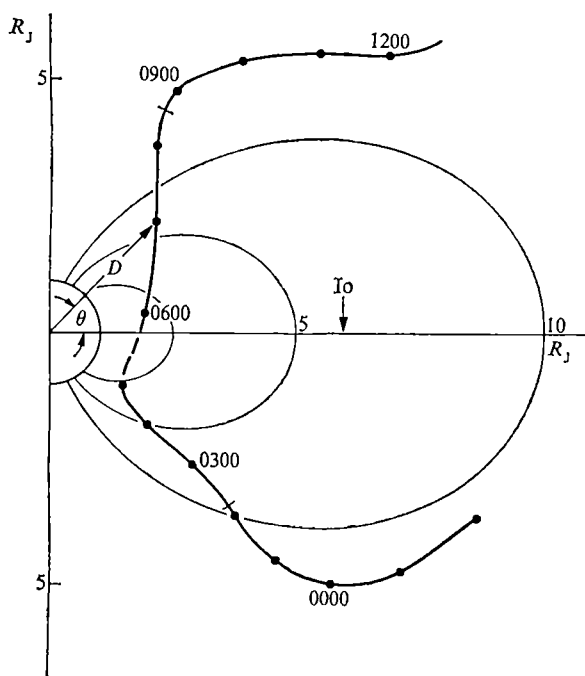


Fig 1 Path of Pioneer 11 spacecraft as function of magnetic latitude, θ , and distance, D , near closest approach to Jupiter, which occurred at 0522 GMT December 3, 1974. Numbers on path indicate time (GMT). Location of orbit of satellite Io is indicated.

222°) of this model, identified at D_2 , yielded a field configuration and intensity which were inconsistent with several earlier independently derived estimates obtained from ground based observations of radio emissions²⁻⁶.

A second opportunity to study the main magnetic field of Jupiter occurred on December 3, 1974, when Pioneer 11 passed within $0.6R_J$ of the planetary surface. The near planet trajectory is shown in Fig 1, presented in a magnetic latitude and distance format using the assumed D_2 model field⁷. The wide excursion in latitude is accompanied by a similarly wide coverage in longitude, extending 660° from $\lambda_{III}=30^\circ$ at 0000 GMT to $\lambda_{III}=330^\circ$ at 1100 GMT.

A high field magnetometer was placed on Pioneer 11 by NASA-GSFC to provide extended range coverage up to 17 gauss, values believed representative of high latitude, low altitude field intensities from radio astronomy observations²⁻⁶. Here we present the preliminary results of an analysis of quick-look data obtained from this instrument. We find that within $3R_J$, the planetary magnetic field is much too complex to be represented by a simple OTD and that higher harmonic multipoles are required.

The instrumentation aboard Pioneer 11 consisted of a single range triaxial fluxgate magnetometer capable of measuring fields up to 10 gauss along each orthogonal axis, and associated electronics. The quantisation step size for the 10-bit A/D converters used is ± 600 gamma for fields less than 2 gauss. An instantaneous vector measurement of the magnetic field is obtained once every three revolutions of the spacecraft (36 s) when a reference axis crosses through the ecliptic. The magnetometer weighs 272 g and uses 0.3 W of power from the GSFC Cosmic Ray Telescope Experiment. A more complete description is given in ref. 8.

The raw magnetic field data are translated to a Jupiter centred spherical coordinate system and combined with spacecraft trajectory information to yield a measurement

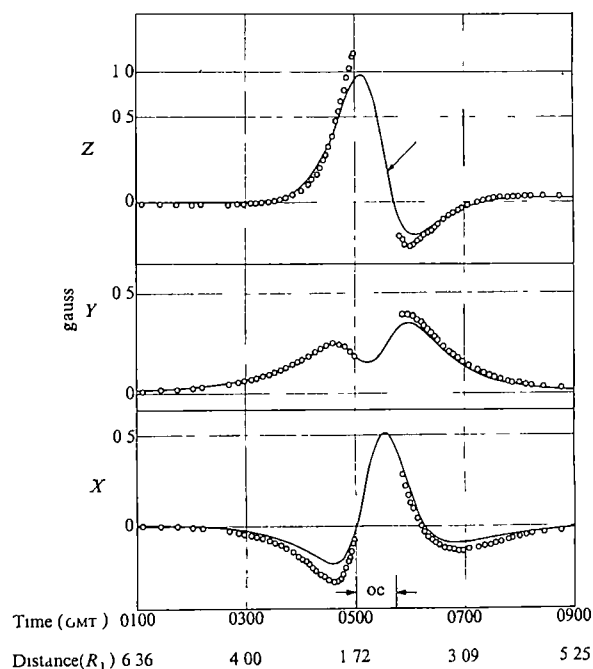


Fig 2 Comparison of expected magnetic field of Jupiter (solid line) assuming simple offset tilted dipole magnetic field model with observations (circles) by GSFC high field triaxial fluxgate magnetometer. Period of occultation of radio transmission of data indicated by OC. Spacecraft-centred inertial cartesian coordinates are used (z axis points to Sun, x axis points to south ecliptic pole).

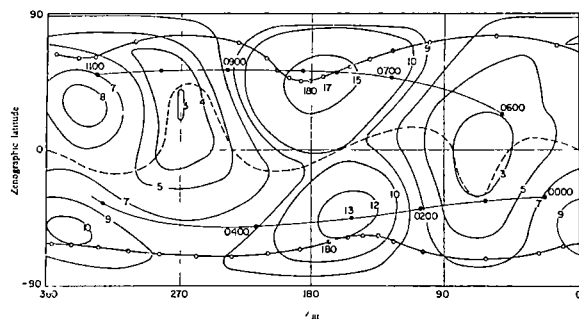


Fig 3 Isointensity contour map of magnetic field on surface of Jupiter predicted by GSFC model O₁, using 15 spherical harmonic coefficients up to octupole moment. Field strength of each contour given in gauss. Trace of sub-Pioneer 11 point, at time (GMT) on 3 December, is indicated accordingly (four digit numbers). For Io footprint, equal 20° longitude increments at Jovigraphic latitude = 0° and radial distance = 5.95R_J were used as starting points. Note significant distortion of field lines from meridian planes. —, Footprint of field line through Io, — — —, sub-Pioneer 11 trace, - · - · -, magnetic equator

set. Conversely, given a magnetic field model, we can obtain the equivalent readings which should be observed in each orthogonal axis of the instrument coordinate system. This is illustrated in Fig 2 where the theoretical digital counts expected from the D_2 model are plotted along the Pioneer 11 trajectory (solid line) and compared to the observations (circles). For radial distances greater than $3R_J$, there is reasonable agreement with the predicted values but closer to the planet, the field magnitudes increase rapidly, much faster than the expected inverse cube law for a dipole. This implies the existence of higher order multipoles, which become very significant for distances smaller than $3R_J$.

The observations between 1.7 and $5.6R_J$ have been fitted in a least squares sense to first, second and third degree spherical harmonic expansions—terms corresponding to a centred dipole, quadrupole and octupole moments. We find a significant decrease in the root mean square (r.m.s.) of the residuals when quadrupole and octupole terms are included. An offset tilted dipole representation, which uses six of the eight dipole plus quadrupole coefficients, yields a vector r.m.s. of 0.026 gauss which is considerably larger than the quantisation step size of the instrument, whereas an octupole expansion, using 15 coefficients, reduces the r.m.s. value to 0.019 gauss. This is a clear indication that the main field of Jupiter is much too complex to be represented by an OTD, especially for $R < 3R_J$.

To illustrate the complexity of the field topology near the planet, the surface field derived from the 15 coefficient spherical harmonic expansion, that is, terms up to and including an octupole, has been plotted in the form of iso-intensity contours as shown in Fig 3. The field strength at the poles is asymmetrical and much larger, 17 and 13 gauss in the north and south respectively, than that obtained from OTD representations. The magnetic equator, as defined by the zero dip angle, is highly distorted with respect to the Zenographic equator. Some caution must be exercised in the interpretation of these results since they represent only one solution of many possible fits to the observations along the spacecraft trajectory. This means that inclusion of higher order multipoles and/or a different data interval lead to a modified coefficient set. Nevertheless, the contribution of higher order multiple terms is evident, independent of the particular expansion used to derive the surface field. Thus, the differing results obtained in separate studies of the radio

emissions may be capable of being resolved in terms of different source locations and the sensitivity of the interpretation to distortion by non-dipole terms.

The iso-intensity surface map in Fig 3 shows many unique features. Most remarkably, the 'footprint' of the field line through the satellite Io passes near both the north and south magnetic dipole regions. So particles mirroring on the L shell of Io also mirror in the Zenovian auroral regions. The hemispherically asymmetrical field leads to uniquely asymmetrical mirror points for such trapped particles. The field intensities in the polar regions are now high enough to allow further elaboration and detailed study of the relationship of decametre emissions by Jupiter and their modulation by Io^{2-6} . The longitude of the north polar region, $205 \pm 15^\circ$, is quite consistent with much prior work²⁻⁶. These four features suggest to us that the source of decametre radio emission from Jupiter is sporadic precipitation of particles into the Zenovian auroral regions. Although the distortion of the magnetic equator is large at the surface, we find that it rapidly approaches a simple OTD model at $R > 2.5R_J$. The dipole terms of the OTD correspond to a dipole moment of 4.2 ± 0.1 gauss R_J^3 at a tilt angle of $8.5 \pm 1.0^\circ$ and a longitude $= 225^\circ \pm 10^\circ$.

The motion of charged particles in such a non-axially symmetric field configuration means that a further complexity is added to the interpretation of such observations. Indeed, it is these asymmetries which also suggest to us that the reason for the 10-h periodicity^{9-12,13} in charged particle observations on Pioneer 10 depends in part on a special azimuthally asymmetric magnetospheric configuration. Thus, a particular field geometry occurs only once every Jovian rotation, rather than the twice which is demanded by any nearly axially symmetrical model such as an OTD with small offset.

We believe that these results and the derived quantitative octupole model of the main magnetic field of Jupiter will permit reconciliation of the previous 20 yr of radio astronomy data with the real magnetic field of Jupiter. Moreover, with the *in situ* charged particle observations by Pioneer 10 and 11, an understanding of the basic source mechanisms and processes leading to the suite of emission phenomena should now be possible.

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New microstructure of decametre solar radio bursts

THE fine structure of a variety of decametre solar radio bursts obtained with high temporal and frequency resolution spectrographs provides valuable information on the generation mechanisms of these bursts together with the physical conditions in the corona. Earlier observations¹⁻¹⁰ of solar radio bursts in the decametre region have revealed some types of fine structure bursts, drift pairs and split pairs being examples of fine temporal and frequency structure bursts respectively.

We report here a new type of solar decametre bursts which, we believe, has been seen for the first time. This was a result of high resolution (frequency ~ 5 kHz and time ~ 10 ms) spectral observations made over a frequency range of only 0.5 MHz near 35 MHz with a scanning rate of 100 Hz. A circularly polarised crossed-Yagi antenna with an effective gain of about 8 db was used to track the Sun. Recording was on a continuously moving 35-mm film.

This high resolution spectroscope, went into regular operation in February, 1974. Since then a large number of solar radio bursts, including a variety of fast drift bursts (individual, in large groups, as well as storm bursts) have been recorded. Three noise storm periods, one each in July, August and September, 1974, were interesting.

Figure 1a and b shows examples of a new class of bursts which occurred respectively on July 17 and August 6, 1974. Each of the bursts, which we call 'complementary bursts' is characterised by two components, the first of which is essentially a fast drift burst comprising patches of strong and weak emissions (or emission gaps). After a time delay of ~ 0.5 s, narrow bands of intense emission are seen to have occurred. They cover a frequency range of ~ 30 –70 kHz which is almost equal to the one corresponding to the emission gap in the first component. Figure 1c shows another event which occurred on July 16, 1974. Here the intense emission in the second component covered a range of ~ 350 kHz against the very weak emission in the first component. Again, the time delay between the two components was ~ 10 s.

Figure 1d shows another example of this type of burst. This event was recorded on September 13, 1974. It is interesting that there are three components in this burst. Furthermore, in this case there is an intense narrow band emission in the second component corresponding to the emission gap in the first component, and corresponding to the emission gap in the second component there is an intense narrow band emission in the third component. These examples show that, in general, the frequency drift rates of these narrow band emissions are less than those of their first components. In particular, the drift rates are higher if the intercomponent time delay is more (Fig 1c).

Thirty four 'complementary bursts' were recorded from July through September, 1974. On average, two different groups of delays between the components of bursts are observed. The duration, at a given frequency, of all the components is ≤ 1 s. Figure 2 is a histogram of the time delay between the components of bursts and the number of bursts. It shows two delay groups centred around 1–2 s and 10–15 s. The emission gap for the first group covers a frequency range of about 30–70 kHz. For the second group it covers a range of about 100–200 kHz. Again, the delayed narrow band emissions of the bursts show frequency drift rates of ≤ 500 kHz s⁻¹ for the bursts with the intercomponent delays of 1–2 s and ≥ 1 MHz s⁻¹ for the bursts with 10–15 s intercomponent delays.

The phenomenon described here could possibly be the result of either the same stream of electrons, or to two or three successive electron streams. In the case of more than one stream, the first stream, while moving out through a smooth

coronal plasma in which the conversion of plasma waves into transverse electromagnetic waves is very poor, might excite only sporadic emissions. Such a stream might create some turbulence behind it. The next stream, which encounters such a turbulence, should give rise to a continuous type III emission which is not what is observed. It is thus difficult to imagine how the intense narrow band emissions in the later components could occur only over the frequency range corresponding to which there are emission gaps in the previous component. The possibility of successive streams was invoked to explain type IIIb associated with type III bursts⁶.

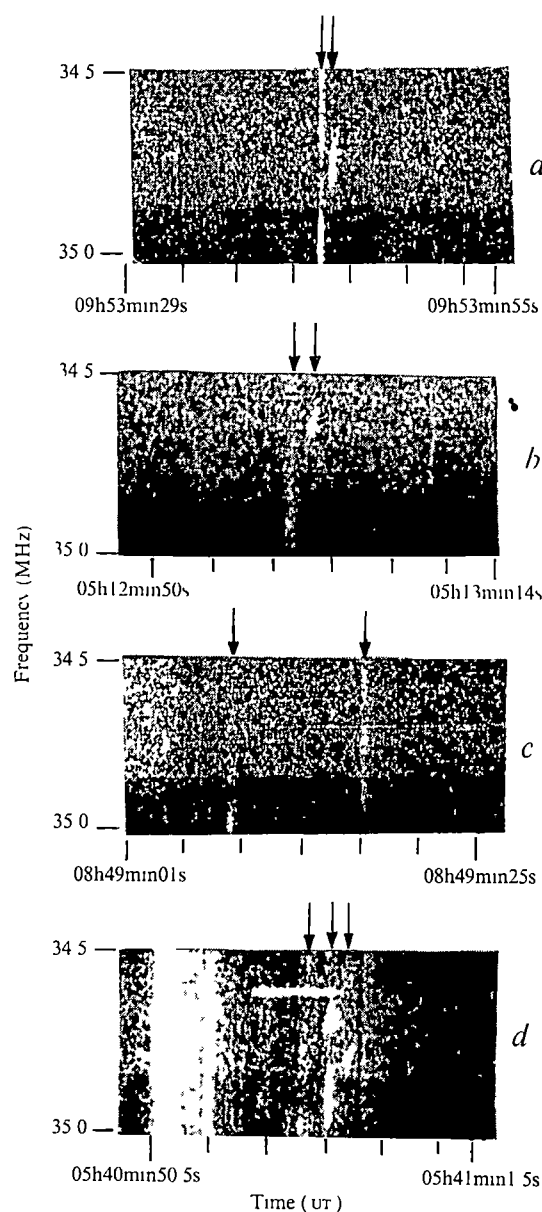


Fig 1 Dynamic spectra of solar bursts near 35 MHz. a, Burst on July 17, 1974 showing two components (marked by arrows above), the first with an emission gap around its centre, the second strong and delayed by ~ 0.5 s. b, Similar event on August 6, 1974. c, Burst on July 16, 1974, the second bright component is delayed by ~ 10 s. d, Bursts on September 13, 1974. The bright vertical patch is a group of type III bursts. The next event shows three components displaying similar characteristics as the two-component events. Horizontal bright line is due to a local transmission. The lower dark strip in each photograph is due to receiver gain variation of ~ 1 db.

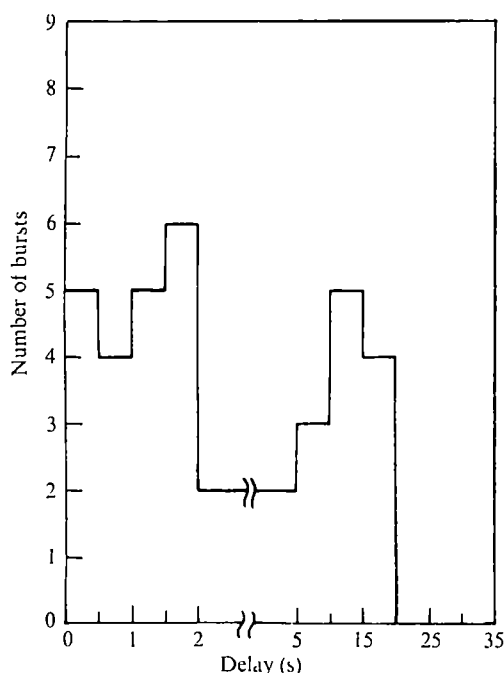


Fig 2 Histogram showing the number of bursts as a function of time delay between their components. Total number of bursts $n = 34$ observed from July through September, 1974. Note the two delay groups, one around 1–2 s and the other around 10–15 s.

On the other hand, if a single stream model is assumed the sporadic emissions of the first component may be due to the sporadic leakage of electrons moving along a coronal streamer. The narrow band emissions in the following components may then occur due to the electrons which propagate at an angle with the streamer. The region where this happens might be the region of a discontinuity at the interaction of the streamer with another coronal structure moving with a different velocity¹¹. The observed slower frequency drift rates of these narrow band emissions then depend on the angle between the direction of propagation of these electrons and the plasma density gradient in these magnetically turbulent regions¹². It is also possible that the narrow band emissions could be the result of transmission characteristics of the propagation medium which acts like a band-pass filter. But this does not explain why the narrow band emission of the second component always corresponds with the emission gap in the first component. We must, therefore, think in terms of duct-like coronal structures which trap and delay part of the electromagnetic radiation of the first component, analogous to terrestrial whistler mode propagation. White light coronagraphs of coronal loop structures lasting for less than an hour and observed over distances from 1.5 to 6 solar radii were made during the Skylab mission¹³. These structures may provide the necessary trapping ducts.

The narrow band emissions in the second component of these bursts are relatively more intense. If these emissions are due to the acceleration of electrons in the coronal structure interacting with the coronal streamer, then one should invoke a process whereby the amplitude of the intensity of these emissions is built up.

We note that these complementary bursts are a distinct class by themselves in the sense that there is no association between them and type III bursts. Clearly this is different from the association between type IIb and type III bursts wherein the former occurs as a precursor to the latter⁶.

The frequency structures that have been reported here as complementary bursts cannot be attributed to the effect of Faraday rotation since our antenna is circularly polarised.

Polarisation observations of this type of bursts would be rewarding. So a provision is being made in the spectroscope for making observations of right and left circular polarisation.

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Storm-time increases in the ionospheric total electron content

CHANGES in the total electron content of the mid-latitude ionosphere during the first day of each of 20 geomagnetic storms have been monitored by Papagiannis *et al.*¹ They have shown that during the positive phase of the storms the magnitude of the increase in total electron content exhibits a very pronounced maximum near sunset. Although Rishbeth and Hanson² have shown that plasma convergence in the F region itself cannot account for the observed increases, an inward meridional $E \times B$ drift of the plasma may compress the H^+ gas at great heights^{1,2}, giving rise to a field-aligned flow of H^+ into the ionosphere. We have investigated the consequences of field-aligned H^+ flow by integrating the F region–protonosphere equations of motion and continuity for O^+ and H^+ along magnetic tubes that are undergoing $E \times B$ drift. The range of integration is from the lower F region to the equatorial crossing point of the magnetic tube, the method of integration being essentially that of Moffett and Murphy³. Our results support the idea that an influx of ionisation from the protonosphere may be partly responsible for some storm-time increases in ionosphere content.

An important feature arising from our investigation is the relationship between the meridional $E \times B$ drift of the plasma and west-east $E \times B$ drift relative to the Earth. This relation results from the assumption of an incompressible magnetic field. Rishbeth and Hanson² and Murphy⁴ have shown that for a dipole field, at the equatorial crossing point of the field line,

$$\nabla v_{em} = 6v_{1eq}/r_{eq} \quad (1)$$

where v_{1eq} is the value of the meridional component of $v_{em} = E \times B/B^2$ at the equatorial crossing point (v_{1eq} being positive in the radially outwards direction) and r_{eq} is the geocentric distance of the equatorial crossing point. The derivation of this expression requires the conditions $\nabla \times E = 0$ and $\nabla \times B = 0$. The first of these arises from conservation of the magnetic field. The second is consistent with the dipole field approximation and is justifiable provided electric currents are small enough. Note that equation (1) is correct even if a west-east drift occurs, as will usually be the case.

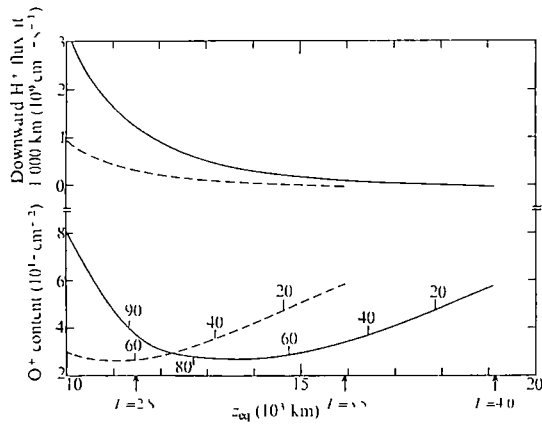


Fig 1 Plots of the O^+ content in a tube with base area 1 cm^2 at 200 km and of the downward H^+ flux through the $1,000\text{-km}$ level as a function of equatorial crossing height, z_{eq} , of the magnetic tube —, Results when $L = 4.0$ initially, ---, results when $L = 3.5$ initially. The plasma temperature at the equatorial crossing point is always $\leq 6,000 \text{ K}$, no account is taken of the increase in temperature that will arise from plasma compression. The numbers on the curves give the local time (min) that has elapsed since the tube started drifting

An alternative expression for ∇v_{em} can be derived by resolving v_{em} in the meridional and west-east directions

$$v_{em} = v_{\perp} \hat{n} + v_{\phi} \hat{\phi} \quad (2)$$

where \hat{n} is a unit vector normal to \mathbf{B} in the meridional plane and $\hat{\phi}$ is a unit vector in the west-east direction, ϕ being the local hour angle and also the azimuthal angle of spherical polar coordinates. At the equatorial crossing point of the field line this alternative expression takes the form^{5,6}

$$\nabla v_{em} = \frac{4v_{\perp}^{eq}}{r_{eq}} + \frac{\partial v_{\perp}^{eq}}{\partial r_{eq}} + \frac{1}{r_{eq}} \frac{\partial v_{\phi}^{eq}}{\partial \phi} \quad (3)$$

where v_{ϕ}^{eq} is the value of the west-east plasma drift velocity relative to the Earth at the equatorial crossing point

Equating equations (3) and (1) gives

$$\frac{\partial v_{\phi}^{eq}}{\partial \phi} = 2v_{\perp}^{eq} - r_{eq} \frac{\partial v_{\perp}^{eq}}{\partial r_{eq}} \quad (4)$$

The significance of equation (4) for our purpose is that a uniform, inward meridional drift (giving a negative v_{\perp}^{eq}) tends to make v_{ϕ}^{eq} negative. A negative value for v_{ϕ}^{eq} means that the plasma is rotating more slowly than the Earth. The time taken by the plasma to drift from a position with a particular hour angle ϕ_1 to a position with a later hour angle ϕ_2 may be much greater than the local time between ϕ_1 and ϕ_2 . If a downward field-aligned flux from the protonosphere is caused by an inward drift, then the effect of this flux on the F region is magnified by the increase of time available for a given observing period of local time.

Equation (4), of course, gives only the rate of change of v_{ϕ}^{eq} , the value of v_{ϕ}^{eq} when the inward drift commences is important. In our calculations we have arbitrarily taken $v_{\phi}^{eq} = 0$ at the start of the inward drift, since indeed a negative initial value for v_{ϕ}^{eq} would increase the local-time effects of the downward H^+ flow.

We adopted a time-independent Jacchia model atmosphere, with an exospheric temperature of $1,000 \text{ K}$. The linear loss coefficient for O^+ at 300 km was $3.2 \times 10^{-4} \text{ s}^{-1}$ and a horizontal

equatorward wind in the neutral air with velocity 150 m sec^{-1} was assumed. The concentration of neutral hydrogen at 400 km was taken to be $10^5 \text{ atoms cm}^{-3}$ and the rate coefficient for H^+-O charge transfer to be $1.4 \times 10^{-9} \text{ cm}^3 \text{ s}^{-1}$ (ref 7, and E E Ferguson, private communication). Typical values of electron and ion temperatures for heights less than $1,000 \text{ km}$ were taken from Brace *et al*⁸ and Evans⁹. We have pointed out (in a paper to be published) that increases in T_e and T_i at great altitudes tend to increase the downwards field-aligned flow of H^+ , and it has been noted by Rishbeth and Hanson² that inward $\mathbf{E} \times \mathbf{B}$ drift will tend to heat the plasma by compression. We have chosen to take, above $1,000 \text{ km}$, T_e equal to T_i and a temperature gradient with height of 2 K km^{-1} until a temperature of $6,000 \text{ K}$ is reached. An estimate of the compression heating shows that this is a very conservative value for the plasma temperature at great altitudes. Possible production of O^+ by photoionisation of atomic oxygen was ignored, so that any increases in O^+ content may be ascribed to influx of H^+ .

In our investigation we examined the behaviour of the plasma in two tubes of plasma. One tube (tube 1) underwent $\mathbf{E} \times \mathbf{B}$ drift from $L = 3.5$ to $L = 2.8$, the other (tube 2) from $L = 4.0$ to $L = 2.8$. In both cases the plasma was given a meridional drift velocity of $v_{\perp}^{eq} = -1 \text{ km s}^{-1}$. This value is based on the work of Park¹⁰. He has interpreted whistler observations of the draining of H^+ from tubes of plasma during and after a magnetic storm as evidence for inward drift of the tubes. The initial value of O^+ content above 1 cm^2 at 200 km was $5.8 \times 10^{12} \text{ cm}^{-2}$ in both tubes. The initial O^+ contents in the tubes were taken to be identical to isolate $\mathbf{E} \times \mathbf{B}$ compression effects from those arising from spatial gradients in electron concentration in the F region that may initially be present. It should be noted that Rishbeth and Hanson² have suggested that concentration gradients in the F region at initial L values of the tubes may contribute to the storm increases seen at lower L values. Our initial H^+ contents were proportional to the volumes of the tubes.

If the tubes start drifting at local time $t_0 = 0$, tube 1 arrives at $L = 2.8$ (the L value of the observations in ref 1) at local time $t_1 = 1 \text{ h}$, whereas tube 2 arrives at $L = 2.8$ at local time $t_2 = 1 \text{ h } 30 \text{ min}$. It is found that at $L = 2.8$ the O^+ content in tube 1 is $2.62 \times 10^{12} \text{ cm}^{-2}$ and in tube 2 $3.72 \times 10^{12} \text{ cm}^{-2}$. Thus an observer at $L = 2.8$ will see during local time $t_2 - t_1 \approx 30 \text{ min}$ an increase of $1.1 \times 10^{12} \text{ cm}^{-2}$ in O^+ tube content. Figure 1 shows the behaviour of the plasma in the flux tubes as a function of L value as we follow the tubes.

This result demonstrates at least that, in discussion of magnetic storm increases, ionisation flow from the protonosphere must be taken into account when inward $\mathbf{E} \times \mathbf{B}$ drift occurs.

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Precambrian geomagnetic field reversal

THE geomagnetic field has reversed many times. Numerous polarity transitions of Cainozoic age, together with very few of Mesozoic and Palaeozoic age, have been studied in detail and the studies indicate that the processes involved have remained relatively unchanged in time. Here we report the first detailed study of a Precambrian reversal.

As part of a palaeomagnetic survey (D K B, M E E, A B Reid, and E W McMurry, unpublished) of Proterozoic sediments in the East Arm Fold Belt of Great Slave Lake, 45 stratigraphically distinct sites have been sampled across a continuous 600-m section. The section consists of steeply dipping, finely bedded, red siltstones in the lower part of the Stark Formation of the Great Slave Supergroup¹. Available K/Ar dates from associated intrusive bodies place the Stark Formation in the age range 1,650–1,800 Myr (ref 1) though palaeomagnetic data may indicate a slightly younger age (D K B, M E E, and E W McMurry, unpublished). Lithologically, the section is very uniform except for many fine green bands which were generally avoided during sampling. Detailed palaeomagnetic studies using thermal and alternating field treatment indicated a marked uniformity in the magnetic properties, all of the specimens are very hard magnetically and possess well defined haematite Curie points.

We have not found any strong evidence that the remanence is primary. It is possible that the red beds could have been remagnetised when they were reheated during the emplacement of the nearby MacKenzie intrusions about 1,200 Myr BP (ref 2). Our observations do not, however, support that idea. The intrusions were emplaced after the folding of the Great Slave Supergroup and the palaeomagnetic pole which we have obtained is 70° from the MacKenzie pole before bedding corrections are applied. As the MacKenzie intrusions represent the most recent igneous event in the East Arm Fold Belt it seems that the red beds acquired their remanence before 1,200 Myr BP. Other workers³ have reached the same conclusion regarding the possible remagnetisation of other red beds in the East Arm Fold Belt. Furthermore, the presence of a reversal and transition zone such as that described here

Fig 1 Stratigraphic profile of thermally cleaned, site mean directional and site mean intensity data for the Stark Formation, after correction for geological dip. Each site is represented by 2, 3 or 4 samples. *a*, Remanent intensity (J), *b*, inclination below horizontal, *c*, declination east of true north. Error bars indicate the standard error of the mean. Dotted line, five-point running mean of J omitting two non-red sites which gave consistent, though weak, results.

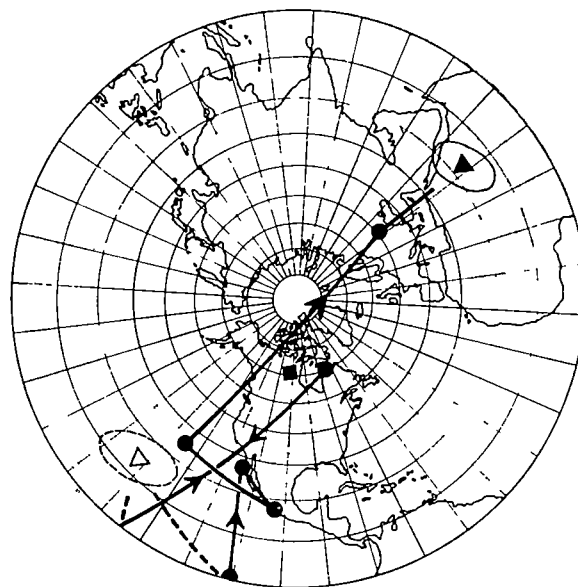
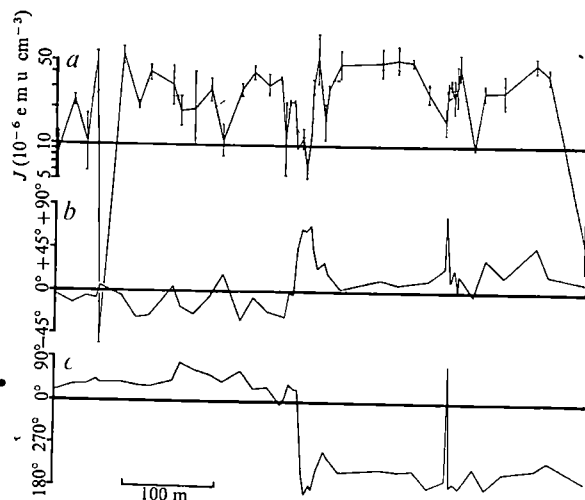


Fig 2 Virtual geomagnetic poles from the Stark Formation after dip correction. Triangles, stable normal (19 sites) and reversed (20 sites) poles with 95% ovals of confidence, circles, intermediate poles. Square is sampling location. Closed (open) symbols and solid (dashed) lines are on the Northern (Southern) Hemisphere. Wulff net.

indicates that secondary components of magnetisation which post-date the initial magnetisation are not significant⁴. We therefore feel that it is reasonable to assume that the magnetisation is primary.

The most prominent features of the thermally cleaned site mean directions and intensities (Fig 1) are the polarity reversal which occurs about mid-way through the section and the apparent field excursion in the lower third of the section. We assume that the remanent intensities are log-normally distributed^{5,6}, and we have shown geometrical means and the associated standard errors. This assumption is, however, not critical as the arithmetical means lead to the same conclusions. Figure 2 shows the corresponding virtual geomagnetic poles (VGPs) for the reversal and excursion, only the overall means for the rest of the section are indicated. A number of features of the reversal are immediately apparent: (1) it occurs across approximately 25 m of section (Fig 1 *b* and *c*), (2) an approximately great circle path is followed by the transitional VGPs (Fig 2), (3) the pre- and post-transitional VGPs are essentially antipodal (Fig 2), (4) the remanent intensity decreases by a factor of 2 or 3 within the transition zone (Fig 1 *a*), (5) the reduction in intensity covers approximately the same time interval as the reversal in direction, though there is a suggestion of reduced but perhaps unstable intensity just above and below the transition zone (Fig 1 *a*).

The field excursion occurs across about 5 m of section approximately 150 m below the reversal (Fig 1). It represents an excursion by the palaeopole of about 80° from the stable direction above and below it, and the VGP of the excursion lies close to the great circle described by the path of the transitional VGPs (Fig 2). There is a suggestion of reduced and unstable remanent intensity in the vicinity of the excursion but the excursion itself does not show reduced intensity (Fig 1).

Accepting the appropriate sedimentation rates given by Kukal⁷ the transition covers 10⁴–10⁵ yr, which implies that either the reversal took considerably longer than the 1,000–2,000 yr observed for more recent transitions⁸ or that the sedimentation rate was a factor of 10–100 higher than expected, or some combination of these two alternatives. The excursion occurred across only 5 m of the section, which—if the sedimentation rate was constant, as is suggested by the uniform lithology—

represents less than 1/5 of the time taken for the transition to occur

The great circle pole path, the antiparallelism of the transition, and the reduced intensity are features common to many but not all of the more recent reversals which have been studied in detail⁹⁻¹⁴. The relationship between the remanent intensity of rocks and the palaeointensity of the geomagnetic field is not necessarily simple. The uniformity of the section both lithologically and magnetically suggests, however, that the coincidence of the reduced remanent intensity with the transition zone is more than accidental. The use of thermally cleaned results in this context is not critical as the uncleaned intensity profile exhibits the same major features.

These observations are relevant to a discussion of the mechanism of geomagnetic field reversal. There are two extreme possibilities: decay and subsequent antiparallel re-establishment of the dipole field, or a 180° dipole 'flip' with no change in strength. The palaeolatitude of the sampling site is 8°, and abundant halite casts offer independent evidence of a tropical climate. At such low latitudes a 'flip' reversal could give rise to a spectrum of intensity profiles ranging from no change to an increase by a factor 2, depending on the meridian followed by the pole. In the present case the transitional pole path passes close to the sampling site and an increase in intensity could therefore be expected. The clear minimum actually observed thus provides strong evidence of a real decrease in the dipole moment during the reversal.

Several workers¹⁵⁻¹⁷ have noted that the period of reduced intensity during recent polarity transitions is longer than the time needed for the direction to reverse, although others have found that these periods coincide¹⁸. It is difficult to place this Precambrian reversal in either class but there is a suggestion of very rapid variations in intensity immediately before and after the change of direction, such as has been seen in more recent reversals¹⁹.

The term 'field excursion' or 'systematic deviation' is usually applied to short duration deviations in pole position, which are greater than 40° or 50° but significantly less than 180° and which are not immediately associated with field reversals²⁰⁻²². The field excursion reported here fits this description but we stress that it is represented by a single site mean. The reduced and/or unstable intensity near the excursion supports findings^{21,23-25} which correlate low latitude poles with reduced intensity.

Perhaps the most significant point about the field excursion is its location near the transition pole path, which suggests that, though it occurred a significant time before the reversal, it is perhaps not wholly unrelated to it. Some coincidence of great circle paths described during several separate reversals⁹ and during distinct field excursions²⁵ has been observed in the recent palaeomagnetic record. The result reported here is consistent with these results and with the hypothesis that field excursions represent aborted reversals²⁰. It also provides support for the existence of long lived non-dipole sources²⁵ or stable equatorial dipoles⁹.

Whatever is the exact mechanism involved in geomagnetic field reversals—dipole decay or dipole flip-over, or a combination of these—the data reported here indicate that the process has apparently remained essentially unchanged for nearly 2×10^9 yr of the Earth's history. The only significant difference involves the possibility that the transition occupied a considerably longer time interval than more recent reversals⁸. These observations place additional limits on the history of the geomagnetic field and, by implication, the thermal evolution of the Earth's core.

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Search for compression before a deep earthquake

DZIEWONSKI and Gilbert¹, using a moment tensor representation of the seismic source, presented evidence for precursive compression before deep earthquakes. For the Colombian earthquake of July 31, 1970 (17 08 05 4 GMT, 1 5° S, 72 6° W, $h > 650$ km), they concluded that a large precursive compression occurred in the focal region, beginning about 80 s before the origin times as determined by ordinary P wave travel times.

These results were, however, obtained from data derived from free oscillations with periods longer than 70 s, and so the time resolution is inevitably limited. Moreover, the phase information contained in the free oscillation data is somewhat obscured by the lateral heterogeneity of the Earth. It is therefore important to search for the existence of precursors by using an independent set of data. Thus, we have examined carefully the direct displacement field caused by a precursor. Specifically, we examined seismograms of the Colombian earthquake of July 31, 1970 and compared these observations with synthetic seismograms of the proposed precursor (Figs 1-4).

The epicentre of the July 31 earthquake is located relatively close to roughly a dozen World Wide Standard Seismograph Network (WWSSN) stations and also close to a station at Naña, Peru. The stations all lie between 6 07° and 16 0° from the epicentre. The wavelength of such a slow precursor is about 1,000 km, and so the Earth can be modelled by a homogeneous medium for our synthetic seismogram computations at these short distances.

For a contraction source with time function $\chi(t)$, the radial displacement in the medium is given² by,

$$u_r = \frac{M}{4\pi\rho\alpha^2} \frac{d}{dr} \left(\frac{1}{r} \chi(t-r/\alpha) \right) \\ = \frac{-M}{4\pi\rho\alpha^2} \frac{1}{r^2} \left[\chi(t-r/\alpha) + \frac{r}{\alpha} \chi'(t-r/\alpha) \right] \quad (1)$$

where α =compressional velocity, ρ =density, r =radial distance, and M =moment of inertia.

The first and second terms in the brackets represent the near-field and far-field displacements, respectively. The time function

of the proposed precursor can be described adequately by the function

$$\chi(t) = \begin{cases} 0 & t \leq -\tau_1 \\ \frac{1}{\tau_1 + \tau_2} \left[t + \frac{\tau_1}{\pi} \sin \frac{\pi}{\tau_1} t + \tau_1 \right] & -\tau_1 \leq t \leq 0 \\ \frac{1}{\tau_1 + \tau_2} \left[t + \frac{\tau_1}{\pi} \sin \frac{\pi}{\tau_2} t - \tau_1 \right] & 0 \leq t \leq \tau_2 \\ 1 & t > \tau_2 \end{cases} \quad (2)$$

where τ_1 is the precursor time constant, τ_2 the time constant of the post-seismic deformation, and $t = 0$ refers to the 'origin time'. This time function avoids discontinuities in displacement, velocity and acceleration at both $t = \tau_1$ and $t = \tau_2$, and represents one of the smoothest time functions for a given time constant. Substituting this time function into equation (1), doubling the result to account for the free surface effect, convolving it with the appropriate instrument functions, and including the necessary cosine factors to account for the azimuth and angle of incidence terms, we obtain the synthetic seismograms. In our present computation we used $M = 5.0 \times 10^{27}$ dyne cm, the value suggested by Dziewonski and Gilbert¹, $\alpha = 8.5 \text{ km s}^{-1}$ and $\rho = 3.5 \text{ g cm}^{-3}$ for the average upper

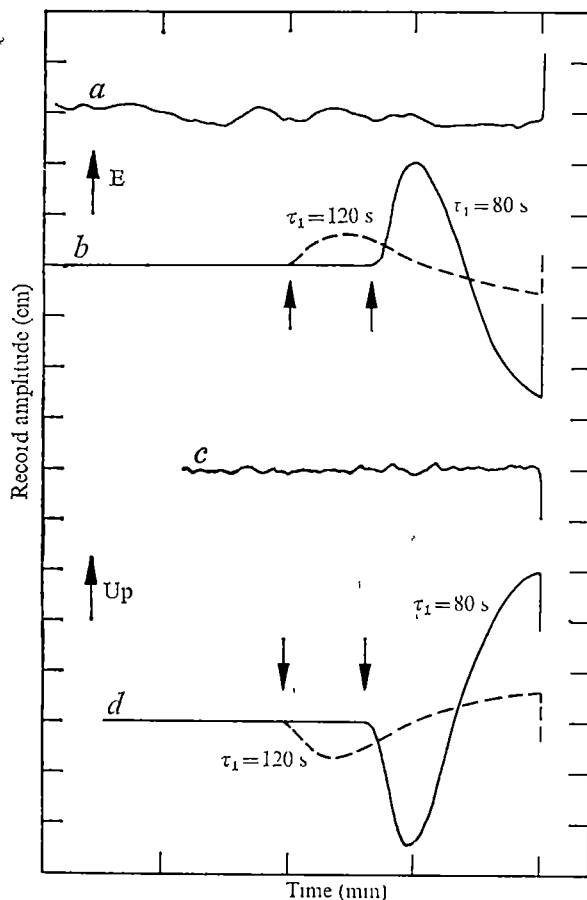


Fig. 1 Long period, WWSSN seismogram from the QUI station (for July 31, 1970) E-W record *a*, observed trace, *b*, synthetic trace. Vertical record *c*, observed trace, *d*, synthetic trace. Arrows next to the synthetic traces (*b* and *d*) indicate precursor onsets. All traces end with the impulsive onset of the normal P wave. Peak magnification = 3,000, $\Delta = 6.07^\circ$.

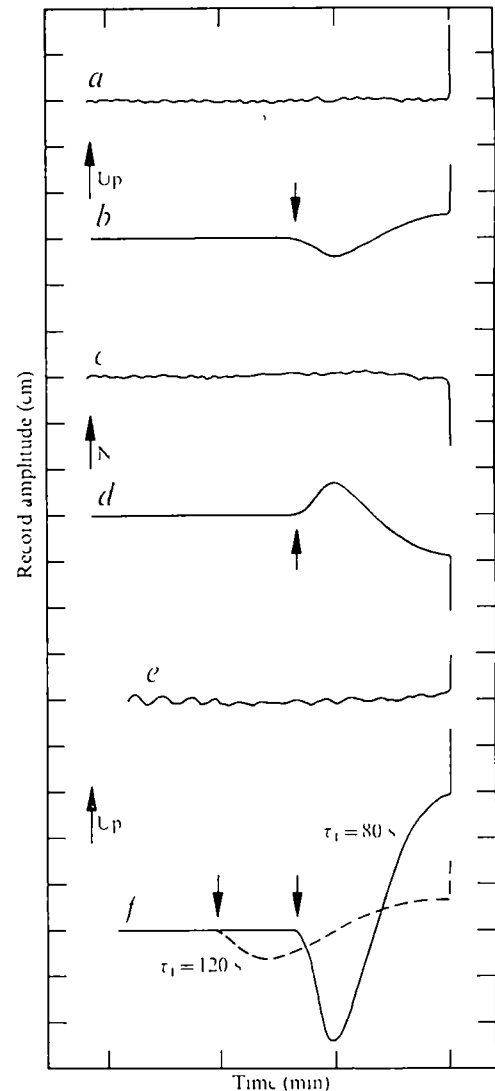


Fig. 2 Long period WWSSN seismograms *a-d*, ARE records. peak magnification = 1,500, $\Delta = 14.94^\circ$. Vertical *a*, observed, *b*, synthetic N-S, *c*, observed, *d*, synthetic BOG records. peak magnification = 3,000, $\Delta = 6.23^\circ$, *e*, observed, *f*, synthetic. Arrows next to the synthetic traces indicate precursor onsets.

mantle. For τ_1 and τ_2 , we consider three cases: (1) $\tau_1 = \tau_2 = 80 \text{ s}$, the value for the precursory time constant suggested in (the abstract of) Dziewonski and Gilbert¹, (2) $\tau_1 = 120 \text{ s}$, and $\tau_2 = 160 \text{ s}$, in this case, $d\chi(t)/dt$ matches the isotropic moment rate tensor suggested by Dziewonski and Gilbert¹ (Fig. 5), and (3) $\tau_1 = \tau_2 = 160 \text{ s}$, this last case represents a more gradual precursor which Dziewonski and Gilbert (personal communication) have suggested might be more appropriate (see Fig. 5).

Long period, vertical seismograms from five WWSSN stations—ARE, BOG, CAR, LPB, and QUI—were available to us. Long period, horizontal records from three of these stations—ARE, LPB, and QUI—were also examined. These seismograms and the corresponding synthetic seismograms are shown in Figs 1, 2, and 3. Recordings of the long period strain network and of the vertical pendulum seismometer at Naña, were also used in our investigations. These records and the respective synthetics are shown in Fig. 4. The very long period information on the strain record indicates tidal strains and some minor surface wave effects from an earlier earthquake in the South Pacific. Neither of these strains was included in the

synthetic strain record The observed strain record has been fitted with a parabola for the 80 min preceding the onset of the main phase which closely approximates the tidal period The synthetics for case (1) predict very large precursor amplitudes, as much as 6 cm peak to peak, on a WWSSN instrument operating at a peak magnification of 3,000 We were, however, unable to identify any trace of such an arrival on any of the

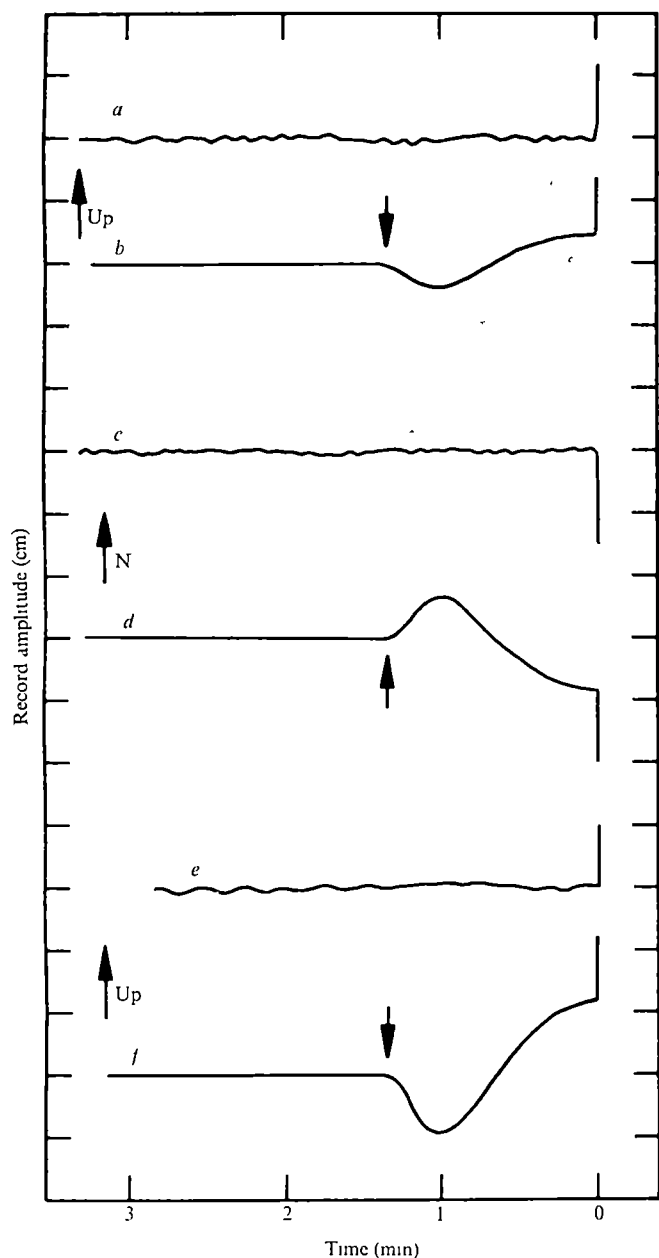


Fig 3 Long period, WWSSN seismograms *a-d*, LPB records peak magnification=1,500, $\Delta=15.61^\circ$ Vertical *a*, observed, *b*, synthetic N-S *c*, observed, *d*, synthetic CAR records Peak magnification=3,000, $\Delta=13.15^\circ$, *e*, observed, *f*, synthetic Arrows next to the synthetic traces indicate precursor onsets

ten seismograms examined Thus, if the precursor time constant is 80 s, the moment must be much less than that assumed here, probably less than 5.0×10^{26} dyne cm For case (2), since the response curve for the Naña strain network is almost flat for $T < 600$ s, the predicted amplitude is reduced only 30% from case (1) (Fig 4A) In the case of the WWSSN instruments, however, we are dealing with the steep portion of the response

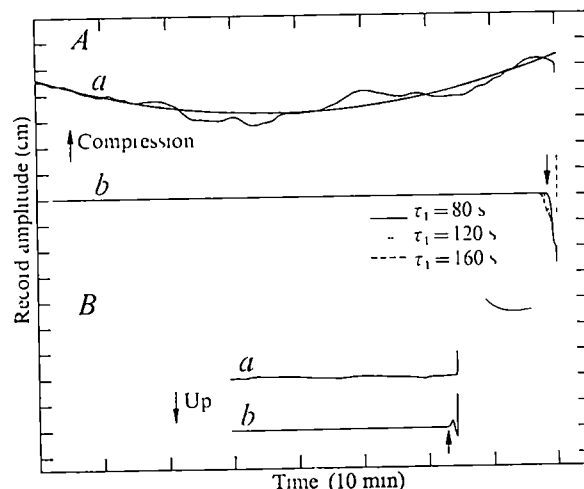


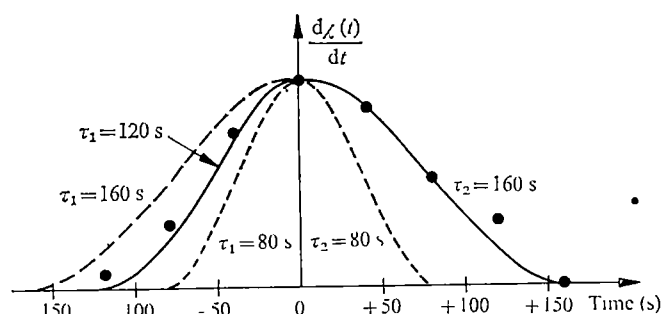
Fig 4 A, Observed (*a*) and synthetic (*b*) strain records for the Caltech north-east strain network at Naña, Peru Tidal effects removed from the synthetic Small arrow indicates precursor onset, $\Delta=11.21^\circ$ Note the change of time scale between this figure and the preceding three B, Observed (*a*) and synthetic (*b*) seismograms for Caltech vertical pendulum seismometer at Naña, Peru Arrow indicates precursor onset, $\Delta=11.21^\circ$ Note that the polarity is reversed

curve (ω^3) and the amplitude is greatly reduced by the increase in τ_1 from 80 to 120 s Nevertheless, two nearby stations, QUI ($\Delta = 6.07^\circ$) and BOG ($\Delta = 6.23^\circ$), still show significant amplitudes (Figs 1 and 2) Thus, on the basis of Naña, QUI and BOG, we may conclude that the isotropic moment must be significantly smaller than the suggested value, probably less than 10^{27} dyne cm For case (3), the precursor signal is almost undetectable at all stations except Naña where the signal is still significant (Fig 4A) If the precursory time is longer than about 200 s, the proposed isotropic precursor would be hardly detectable This time constant is somewhat longer than that suggested by Dziewonski and Gilbert¹, but in view of the discrete time interval of about 40 s used by them it might be within the permissible range

We conclude that if the precursor time constant is less than 120 s, the isotropic moment must be less than 10^{27} dyne cm or, if the precursor moment is 5.0×10^{27} dyne cm, the lower bound of the precursor time is 200 s

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Fig 5 ●, Moment rate tensor ($d\chi/dt$) data of Dziewonski and Gilbert¹ The solid and dashed curves indicate the various moment rate tensors used in this study



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DRS DZIEWONSKI AND GILBERT REPLY—We have stated that for two deep earthquakes the hypocentral region begins to experience compression about 80 s before the origin time determined from the first arrivals of P waves. This statement was based on an estimate of the time at which the isotropic moment rate begins to exceed the noise level visible in the deviatoric part of the moment rate tensor (Fig 2 of ref 1). The figure shows that isotropic moment rate is other than zero at times earlier than -80 s, and obviously our estimates of the "beginning" time could not have the meaning that Hart and Kanamori assigned to it.

Our measurements were carried out in the frequency domain. Transformation to the time domain required certain arbitrary, although clearly stated, assumptions because the spectrum was incomplete and contained noise. In these circumstances, if one attempts to introduce an analytical source function, the matching of the parameters should be done in the frequency domain. For the source function proposed by Hart and Kanamori² and the isotropic moment rate spectrum shown in Fig 1 the best match is for a time constant of 160 s, a time constant of 200 s is also consistent with the data if M_0 is allowed to increase by 50%. In either case the amplitudes of the synthetic seismograms would approach the noise level (the amplitudes would decrease by a factor comparable to that resulting from the increase of a time constant from 80 to 120 s).

It is disappointing that the result of the experiment by Hart and Kanamori is inconclusive, because it seems that with the present instrumentation it may be impossible to

confirm by observations in the time domain the occurrence of precursory phenomena such as those described in our paper¹, the Colombian earthquake was the largest event in its depth range during the past 12 yr. Such confirmation would be very important, as at the present time we must rely on the assumption that the observed spectra should be smoothly interpolated to zero frequency. If this assumption were not valid, then compression might not be precursive. The most important result of our paper¹, which is not subject to this assumption, is that there are volume changes associated with deep earthquakes and that their time function must be significantly different from that of the deviatoric (shear) moment tensor.

In the paper by Hart and Kanamori² we notice a long-period trend in the observed seismograms shown in Fig 1a and c and Fig 2c and e that agrees with the sign predicted by the theoretical calculations, that is, is consistent with the hypothesis of precursory compression. The same is true for a deflection on the strainmeter recording that occurred just before the earthquake (The steeply rising pulse immediately following $t = -\tau_1$ on the theoretical seismograms is determined by the properties of the source function at $t = -\tau_1$. This effect could be substantially reduced if Hart and Kanamori had chosen a function that has a more gradual rise.) Even though none of these examples is significant by itself, the fact that there are several of them is intriguing, particularly as there are no adverse cases.

¹ Dziewonski, A. M., and Gilbert, F., *Nature*, **247**, 185 (1974)

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Chemistry and origin of phlogopite megacrysts in kimberlite

STUDIES of the megacrysts occurring in kimberlite have hitherto neglected the mica megacrysts. We have analysed mica megacrysts from one Lesotho and twelve South African kimberlites. The megacrysts, which vary in size from 4 mm to 3 cm, were taken from both 'basaltic' and micaceous kimberlites, and distinguished from the matrix phlogopites by (i) their greater size, (ii) their dark brown, rather than bronze or light brown, colour, (iii) their rounded shape and corroded margins, (iv) distortion of the {001} planes (in some, but not all, megacrysts), and (v) absence of inclusions of matrix phases such as perovskite and spinel which often occur in the matrix micas.

The average and range of compositions of nine selected megacrysts are shown in Table 1, together with the averages of analyses for primary and secondary micas in peridotite xenoliths in kimberlite (ref 1 and new data) and analyses of micas from four glimmerite nodules in kimberlite (three from the Bultfontein Mine, one from the Wesselson Mine). All the analyses were made by electron microprobe techniques, of which details will be given elsewhere. Nineteen megacrysts were analysed and checked for homogeneity at five or more points, the nine selected megacrysts were homogeneous, or nearly so, whereas the others showed variations of 5% or more in Fe. Inclusion of the other ten megacrysts does not affect the conclusions.

In Fig 1, we plot TiO_2 against Cr_2O_3 for all our megacrysts and new data on micas from four peridotites, together with comparative data for ten peridotite micas given by Carswell¹. Carswell had distinguished between primary and secondary micas in peridotites by virtue of lower TiO_2 , Cr_2O_3 , Al_2O_3 , MgO and Na_2O , but higher SiO_2 in the primary micas. Our data for primary micas from peridotite fall very close to a field drawn from Carswell's analyses, whereas those for secondary micas extend towards higher TiO_2 but maintain the high Cr_2O_3 content. The same two oxides are shown for micas from the four glimmerite nodules. The lines AA' and

Fig. 1 Comparison of the observed moment rate spectrum with the spectra predicted by equation (2) of Hart and Kanamori² for several values of the time constant τ ($\tau = \tau_1 = \tau_2$)

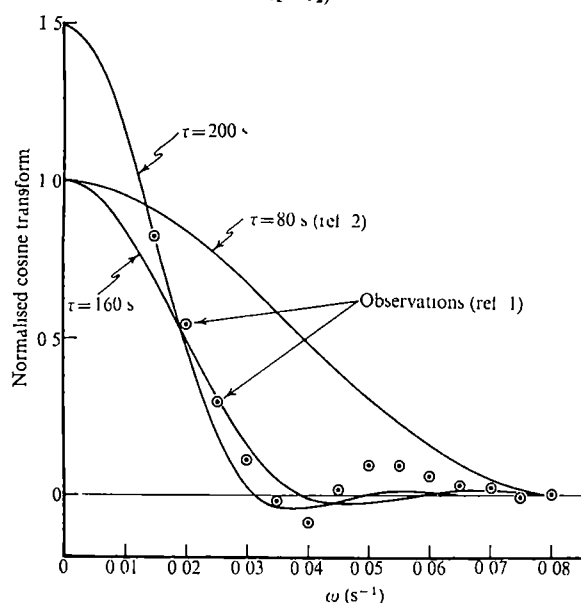


Table 1 Analyses of micas

	1	2	3	4	5	6	7	8	9	
SiO ₂	40.2-42.2	(41.3)	43.4	43.2	44.1	42.4	42.0	38.7	41.3	39.3
TiO ₂	0.6-1.5	(1.06)	1.92	1.09	2.34	1.17	0.53	2.90	0.32	1.11
Al ₂ O ₃	11.2-12.7	(12.1)	9.76	10.5	8.55	10.3	11.6	15.0	12.6	14.9
Cr ₂ O ₃	0.02-0.63	(0.30)	0.25	0.19	0.17	0.31	0.68	1.38	0.70	1.51
FeO	2.9-7.6	(4.9)	7.58	6.08	8.97	6.78	2.56	4.80	2.77	3.06
MnO	0.00-0.03	(0.01)	0.02	0.04	0.03	0.03	0.01	0.02	0.02	0.07
MgO	23.4-25.1	(24.3)	21.7	24.7	20.5	24.3	26.1	22.3	26.8	24.9
NiO	0.03-0.18	(0.11)	0.12	0.23	n.d.	n.d.	0.25	0.12	n.d.	n.d.
CaO	n.d.	n.d.	n.d.	0.00	0.08	0.02	0.00	0.03	0.02	0.03
Na ₂ O	0.05-0.26	(0.16)	0.03	0.14	0.04	0.14	0.18	0.28	0.74	1.22
K ₂ O	10.0-10.6	(10.4)	10.4	9.6	10.5	10.1	10.6	9.10	9.35	8.37
Total	93.3-95.4	(94.6)	(95.2)	(95.7)	(95.2)	(95.5)	(94.5)	(94.7)	(94.4)	(94.4)
mg	0.845-0.939	(0.898)	0.836	0.879	0.803	0.865	0.949	0.893	0.945	0.931
Na/K	0.007-0.038	(0.024)	0.004	0.023	0.006	0.021	0.025	0.046	0.123	0.229

1, Range and mean of nine megacrysts from South African kimberlites (Hololo, Dutoitspan, Wesselton, Star, Monastery, Roberts Victor, Klipfontein, Weltvreden) selected from 19 megacrysts on the basis of chemical homogeneity

2, 3, 4, 5, Glimmerites from Wesselton (No 1083-2), Bultfontein (No 1158, with ilmenite), Bultfontein (No 1159, with amphibole and clinopyroxene), Bultfontein (No 1160, with amphibole and rutile)

6, Mean of two primary micas in ilherzolite xenolith 1141 from Bultfontein and spinel ilherzolite xenolith from De Beers 1197

7, Mean of two secondary micas in chromite harzburgite (1126, Jagersfontein) and amphibole harzburgite (1368, Monastery)

8, 9, Means of five primary and five secondary micas, respectively, from garnet ilherzolite xenoliths in South African kimberlites (from Carswell¹)

BB' at Cr₂O₃ = 0.5 and 1.1 wt % are tentatively chosen to separate the primary and secondary peridotite micas from each other and from the glimmerite micas. For the megacrysts, several features emerge

(i) None of the megacrysts falls within the range of secondary peridotite micas

(ii) Four of our megacrysts fall within, or very close to, the field or primary ilherzolite micas, and hence might be interpreted as being derived by fragmentation of peridotites containing primary micas

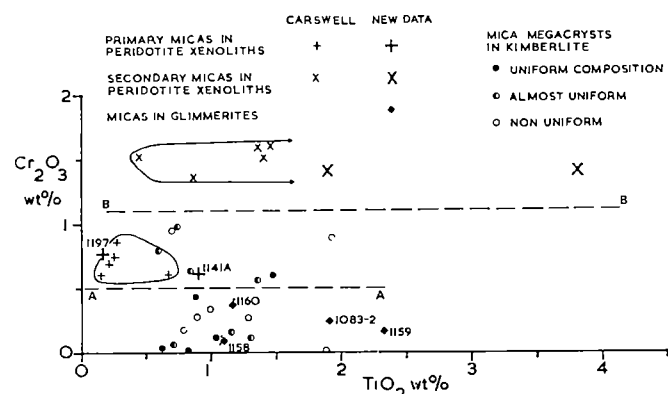


Fig 1 TiO₂ against Cr₂O₃ for micas. The primary and secondary micas from peridotite xenoliths in South African kimberlites, as selected by Carswell¹, are ringed by solid lines. New data for peridotite xenoliths are keyed by numbers that refer to analyses in Table 1. New data for glimmerite micas are ringed by a broken line and keyed to Table 1. All other specimens are megacrysts in kimberlite of which the average and range are listed in Table 1.

(iii) Three of the megacrysts have similar Cr₂O₃ contents to the primary peridotite micas but higher TiO₂ values, perhaps the field for peridotite micas extends to these higher values of TiO₂, or perhaps TiO₂ was introduced from the host kimberlite

(iv) Most megacrysts contain less than 0.5% Cr₂O₃, which serves to distinguish these megacrysts from the peridotitic micas and the megacrysts with peridotitic affinities. In addition, the TiO₂ tends to be higher

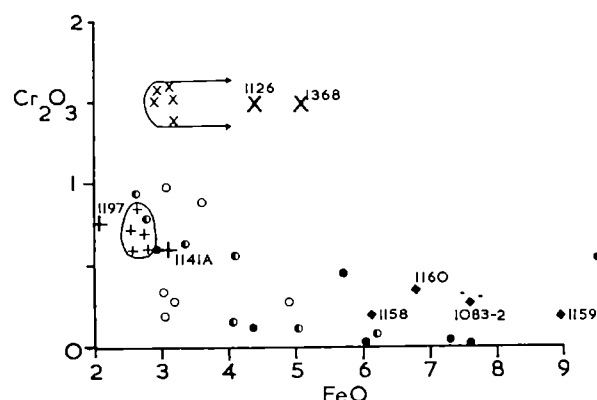
(v) Two of the four glimmerite micas fall directly within the group of 'non-peridotite' megacrysts whereas the other two, although having a higher TiO₂ content than the non-peridotite megacrysts, resemble them in their low Cr₂O₃

values. Here there is the indication of an affinity between the non-peridotite megacrysts and the glimmerites, suggesting that either the megacrysts result from disruption of glimmerite or the glimmerites are accumulations of non-peridotite micas

A further chemical distinction can be made between the peridotite micas and most of our megacrysts. In Fig 2, where Cr₂O₃ is plotted against FeO for the same micas, it can be seen that, in addition to the earlier Cr₂O₃ distinction, there are differences in the total FeO contents. Whereas all the primary ilherzolite micas (and possible 'peridotitic' megacrysts) contain <3.7% FeO, only three of the megacrysts with low Cr₂O₃ contain <3.7% FeO. The other ten non-peridotite megacrysts have a wide range of higher FeO values from 4.04 to 7.62 wt % FeO. The glimmerite micas also have considerably higher FeO values than the peridotite micas

In these chemical features (relatively high FeO and TiO₂, and lower Cr₂O₃) that distinguish them from the peridotitic micas, the non-peridotite megacrysts resemble other megacrysts (often referred to as 'discrete nodules') of pyrope garnet, diopside and olivine found in kimberlite; these are distinct from the broadly similar phases in peridotites in the upper mantle because of their large size, their relatively high FeO and TiO₂ content, and their comparatively low amounts of Cr₂O₃ (refs 2-4). In addition our suggestion that the glimmerites may be accumulations of non-peridotite megacrysts and thereby linked in with the overall megacryst suite, is strengthened by the fact that diopside occurring as a minor phase in one of the glimmerites (BD1159, Bultfontein Mine) is a high FeO/low Cr₂O₃ variety that chemically resembles the diopside 'discrete nodules' occurring in kimberlite. The

Fig 2 FeO against Cr₂O₃ for micas. Ornament as in Fig 1



megacryst suite as a whole, in its relatively high FeO and TiO₂, but lower Cr₂O₃, contents seems to be strongly tied in with the kimberlite event when FeO and TiO₂ (together with many other minor and trace elements) were strongly concentrated relative to presumed parental peridotite⁵

In summary, two distinctive groups of phlogopite megacrysts have been recognised, namely chromium-rich peridotite-derived micas and titanium-rich kimberlite megacrysts. The distinction between the two is important when using the megacrysts for dating purposes as an analysis of a sample consisting of indiscriminated megacrysts would yield a meaningless, hybrid age. In addition, in models for the genesis of kimberlite by fractionation of high pressure phases (the discrete nodule suite) from a primitive ultrabasic parent magma, phlogopite fractionation will have to be taken into account, in addition to that of garnet, pyroxene and olivine.

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Flow separation in meander bends

It is generally assumed that downstream flow through meander bends is helicoidal and is accompanied by a transverse, bottom flow component directed towards the inner bank¹⁻³. Thus particles of sediment on a point bar are transported at some angle inwards from the generalised local downstream flow vector^{4,5}. As pointed out by Bagnold², however, "a stage

must be reached at which the flow along the inner boundary becomes unstable and breaks away from the boundary, leaving an intervening space occupied by a zone of unstable and confused motion". The experimental results of Leopold *et al.*⁶ leave no doubt that this phenomenon of flow separation (Fig. 1) can be a highly important feature in river hydraulics. Since 1960, however, no workers have extended these initial experimental ideas into field situations. Existing models of sedimentation and erosion in sinuous channels^{4,5} ignore any possible effects of flow separation. Here we draw attention to examples of flow separation in natural meander bends and attempt to define an empirical criterion for predicting the onset of separation.

Flow separation in meander bends is best expressed as a function of bend tightness and Froude number⁶. Bend tightness is most usefully defined as the dimensionless ratio between meander radius (R_m) and flow width (w) (Fig. 2). This ratio should represent a legitimate scaling factor for both small and large channels since both width and radius bear approximately linear relationships to channel magnitude expressed as meander wavelength⁷. Use of the Froude number (dimensionless) as the other variable allows results from a wide range of channels to be compared.

Our results for the occurrence of flow separation in natural meanders were obtained from intertidal meandering channels in the Solway Firth, Scotland⁸. These are cut into interlaminated muds and silts, and are suspended-load channels according to the terminology of Schumm⁹. Four meander bends were studied, observations and measurements (23 in all) being made during several ebb tides. A wide range of R_m/w Fr values were obtained (see Fig. 2). The results indicate that flow separation is possible in a range of meanders at quite modest Froude numbers (0.27–0.42). Reaches showing flow separation are well separated from those which do not. The discriminant curve drawn indicates that flow separation is a steeply decreasing function of Froude number as the meander bend becomes less tight. The discriminant curve has been extrapolated to the left in Fig. 2 to take in the experimental and field data of Rozovski³, where no flow separation was noted, and the field data of Taylor *et al.*¹⁰, where flow separation was observed. In the latter case the Froude number for the flow was quoted only as less than 0.1. Figure 3 shows a replot of experimental

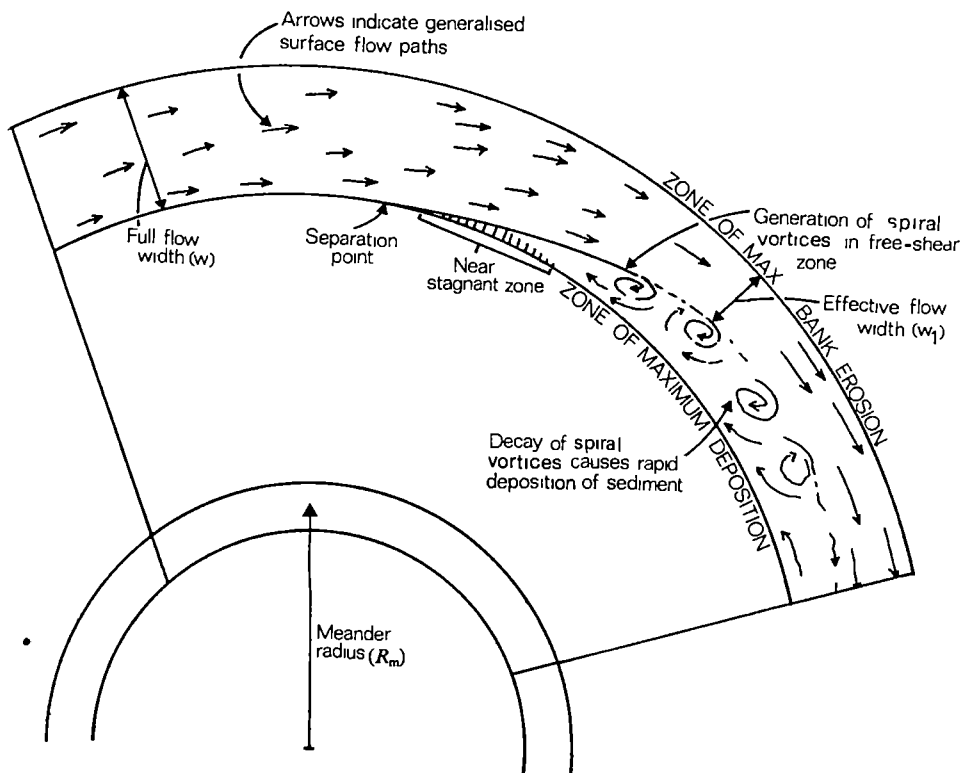


Fig. 1 Sketch to show the main characteristics of separated flows in meander bends

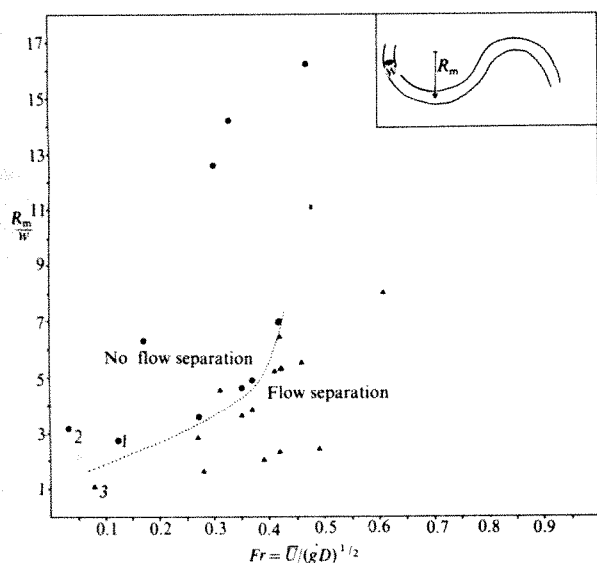
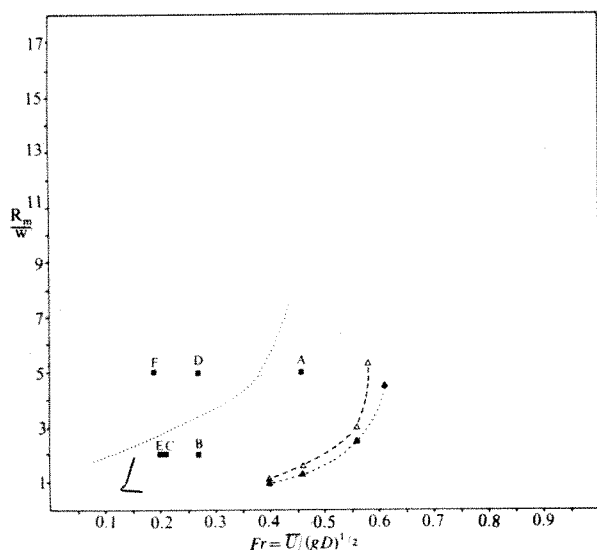


Fig. 2 Dimensionless graph to show the occurrence of flow separation in natural channels. Data points 1 and 2 refer respectively to experimental and field results (R. Desna) showing no flow separation (ref. 3). Data point 3 refers to the River Barwon (ref. 10) where flow separation was observed but where the Froude number is given only as less than 0.1. ●, No flow separation; ▲, flow separation; R_m , meander radius; w , flow width; U , mean velocity; D , flow depth.

data for the onset of spill resistance⁶ as the flow in meander bends becomes locally critical. This plots sensibly to the right of the discriminant curve of Fig. 2 and follows the same general trend. Comparison of field and experimental data confirms that flow separation in meanders is not confined to flows which exceed the spill resistance threshold.

The effects of flow separation in our study reaches were: (1) a decrease in the effective width downstream and opposite the separation point of up to 50%, thus greatly increasing the local velocity and hence the erosion rate for the outer bank (Fig. 1); (2) rapid deposition of suspended silt in the inner

Fig. 3 Dimensionless graph showing the discriminant curve of Fig. 2 (---; depth range = 50–350 mm, Solway Firth) together with curves marking the onset of spill resistance in experimental channels⁶. ▲, Data of ref. 6 for onset of spill resistance in experimental meandering channels (flow depth = 27 mm); ▲, same for channels 20 mm deep. Note the depth effect in the latter curves. Also shown are Allen's model channels⁴ used in his theoretical study of deposition in meander bends (■ (A–F)). We predict that channels A, B, C and E should show flow separation at bankfull discharges.



separation zone (Fig. 1) and development of upstream-facing current ripple bedforms. The boundary between the main downstream channel flow and the more sluggish fluid in the separation zone periodically deforms into spiral vortices. These decay as the separation zone merges back into the general downstream flow at the meander inflection point. The decay and inward movement of eddies associated with these vortices is the cause of the high deposition rates of suspended sediments noted above (up to 20 mm in one ebb/flood cycle).

Our results suggest that flow separation is to be expected in many natural rivers. Of particular interest is the role of the separation eddy in local upstream sediment transport and bedform migration. Such features have been but rarely noted. Their development in the Rivers Mississippi¹¹ and Barwon¹⁰ suggests that flow separation is not restricted to small channels and that the plot in Fig. 2 may be sufficiently valid to warrant general application. Further measurements in natural rivers are needed to confirm and extend the present work.

A further consequence of flow separation is that theoretical models of deposition on point bars that assume a bar-hugging downstream flow cannot be valid for all natural channels. Allen's model channels⁴ are plotted in Fig. 3 and comparisons with our results suggest that several of these should show flow separation during bankfull discharges. This effect might give rise to suites of bedforms and gradations in grain size not predicted by the theoretical analysis.

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Experimental observation of Abraham force in a dielectric

MINKOWSKI's momentum-energy tensor¹ for material media was severely criticised by Einstein and Laub². The density force on matter according to these authors is:

$$\mathbf{f}_M = \rho \mathbf{E} + \rho \mathbf{v} \times \mu_0 \mathbf{H} + (\mathbf{P} \cdot \nabla) \mathbf{E} + (\mathbf{M} \cdot \nabla) \mathbf{H} - \frac{1}{2} \nabla (\mathbf{E} \cdot \mathbf{P} + \mathbf{H} \cdot \mathbf{M})$$

$$+ \mu_0 \frac{\partial \mathbf{P}}{\partial t} \times \mathbf{H} + \epsilon_0 \mathbf{E} \times \frac{\partial \mathbf{M}}{\partial t} - \frac{\partial}{\partial t} (\mathbf{D} \times \mathbf{B} - (1/c^2) \mathbf{E} \times \mathbf{H}) \quad (1)$$

$$\mathbf{f}_{E-L} = \rho \mathbf{E} + \rho \mathbf{v} \times \mu_0 \mathbf{H} + (\mathbf{P} \cdot \nabla) \mathbf{E} + (\mathbf{M} \cdot \nabla) \mathbf{H}$$

$$+ \mu_0 \frac{\partial \mathbf{P}}{\partial t} \times \mathbf{H} + \epsilon_0 \mathbf{E} \times \frac{\partial \mathbf{M}}{\partial t} \quad (2)$$

with $\mathbf{D} = \epsilon_0 \mathbf{E} + \mathbf{P}$, $\mathbf{B} = \mu_0 \mathbf{H} + \mathbf{M}$ and using SI units.

Abraham³ assumes a different density force which agrees with equation (2) in the terms depending on time derivatives.

For an isotropic and homogeneous material the relationship between Abraham and Minkowski density forces is⁴:

$$\mathbf{f}_A = \mathbf{f}_M + [(\epsilon_r \mu_r - 1)/c^2] \partial(\mathbf{E} \times \mathbf{H})/\partial t$$

the last term (the 'Abraham term') has been the subject of a long and difficult theoretical controversy. Although some experiments, such as those described in refs 5 and 6, seemed at first glance to favour Minkowski's point of view, they could equally well be explained by Abraham's tensor⁷.

A direct experiment has been suggested by Marx and Gyorgyi⁸ to verify the reality of the Abraham term. This has never been carried out, to our knowledge, because of the smallness of the expected effect. Pauli⁹, Møller⁹ and others seemed to be pessimistic regarding the possibility of deciding this annoying theoretical discrepancy by a direct experimental check. But our experiment, a refined version of the one suggested by Marx and Gyorgyi, has conclusively proved that the Abraham term not only exists but is measurable with a reasonable degree of accuracy. This finally resolves the old question in favour of Einstein-Laub and Abraham.

The principle of the experiment was as follows: a disk of barium titanate, of dielectric constant near to 4,000, had a small central hole and the edges coated with aluminium to act as a cylindrical condenser. A long tungsten fibre, 0.2 mm in diameter, held the ceramic disk as a torsional pendulum between the two poles of an electromagnet having a vertical uniform magnetic field of about 10 kgauss in the 3.5-cm air gap in which the disk was suspended. The outer edge of the disk was grounded to the bottom pole of the magnet with a vertical very thin strip of gold which did not sensibly alter the mechanical behaviour of the torsional pendulum nor add any measurable Lorentz force. A focused laser provided a 10-m optical arm to amplify the damped oscillations of the torsional pendulum.

In order to synchronise the applied voltage to the torsional oscillations a signal was obtained from a photo-resistor activated each time the light beam crossed the scale. A sinusoidal voltage was generated with peak value 150 V. This voltage was then applied across the dielectric and could be set in phase or in antiphase with the torsional oscillations.

The mechanical force acting on the disk, according to Abraham, would be given by $(\partial \mathbf{P}/\partial t) \times \mu_0 \mathbf{H}$ where \mathbf{P} is the polarisation of the dielectric. In our case the corresponding torque amplitude was about 2.5×10^{-10} N m. According to Minkowski there would be no torque.

The first problem to be overcome in carrying out the experiment was the reduction of noise, occasioned by vibration in the laboratory, to an acceptable level. This was accomplished by mounting the entire apparatus on a platform weighing about 1 ton which was suspended from the roof by a cable which included a damping element in the form of an automobile tyre. The light beam was broken into four paths by mirrors and the final scale was mounted near the apparatus on the same platform. The periodicity of the support system was about 20 s whereas it was only 2.9 s for the torsional pendulum, so there was no noticeable coupling. The most annoying difficulty was that electrostatic forces appeared between static charges forming on the conductors feeding the condenser and their images in the poles of the electromagnet. These were reduced to an acceptable level by allowing an adequate gap between the ceramic and the poles and reducing the alternating applied voltage. With 300 V (peak-to-peak) the total system noise (electrical plus mechanical) gave a deflection of the light spot on the scale of about 1 cm.

The expected stationary amplitude attributable to the Abraham term is about 3.7 cm. In fact, after 1 h of transient, we measured a steady amplitude of (4 ± 0.5) cm. The effect has been checked by reversing the magnetic field and, in other cases, by changing from phase to antiphase the application of the alternating voltage. The observed changes in the transient oscillation agreed, within the error limits, with the predicted values of Abraham's density force.

In order to obtain a more accurate measurement of the Abraham term we are refining the experiment, adding a vacuum system which should reduce damping by about a factor of 4.

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Thermal effects in the necking of thermoplastics

THE new technique of thermovision has been used to study deformation and fracture processes in polymers^{1,2}. This technique provides a thermal image of the polymer surface, taken by means of natural infrared radiation in the wavelength range 2 to 6 μm . The method also allows an estimate to be made of the surface temperature of a polymer, although the value obtained may sometimes be low because of transparency in the polymer films.

Following our first experiments¹, we recently made a film to demonstrate the formation of hot areas during polymer deformation processes. During this work we observed an apparently novel feature of the normal necking process, using rigid poly(vinyl chloride) and polycarbonate plastics. At a moderately high strain rate (10.0 h^{-1}) we found that with PVC only one end of the necked region of the polymer was heated, that is, all the 'cold' drawing was taking place at one point (Fig. 1), whereas with polycarbonate drawing was distributed between the two ends of the necked region (Fig. 2).

Fig. 1 Thermovision photograph of the necking of PVC. Estimated minimum temperature rise was 9°C .

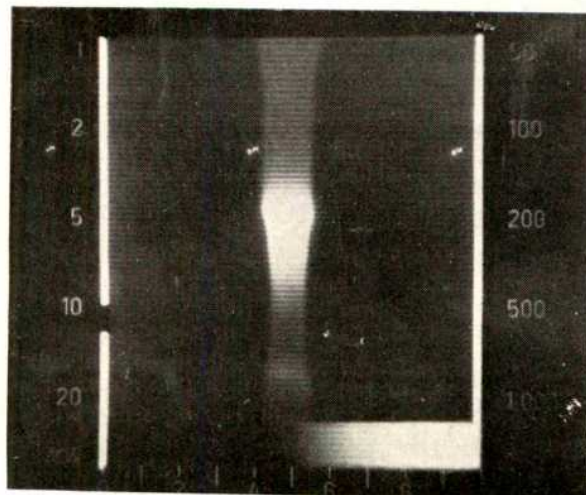
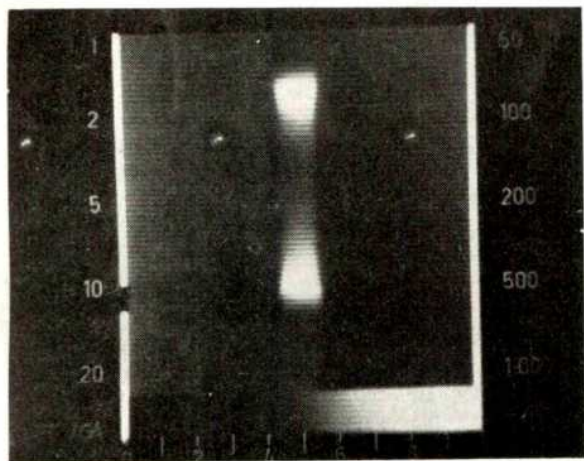


Table 1 Temperature and strain rate sensitivity of the drawing stress σ_D of PVC and polycarbonate plastics

	PVC	Polycarbonate
$\frac{d\sigma_D}{dT}$	(Temperature sensitivity) $-0.71 \text{ MN m}^{-2} \text{ per } ^\circ\text{C}$	$-0.25 \text{ MN m}^{-2} \text{ per } ^\circ\text{C}$
$\frac{d\sigma_D}{d(\ln \dot{\epsilon})}$	(Strain rate sensitivity) 1.5 MN m^{-2}	1.0 MN m^{-2}
Glass transition temperature	67°C	160°C

We suggest the following explanation of this difference in behaviour. With PVC the fall in stress caused by an increase in temperature at the neck greatly exceeds that which would be achieved by the halving of the strain rate which occurs when yielding takes place at each end of the necked region. Thus the reduction in heat losses because of the higher strain rate when

**Fig. 2** Thermovision photograph of the necking of polycarbonate. Estimated minimum temperature rise was 6°C .

straining occurs only at one end dominates the strain rate effect and the plastic strain process becomes localised at one end of the test piece. This explanation is supported by the data given in Table 1; the temperature sensitivity of the drawing stress for PVC is greater than for polycarbonate, whereas the strain rate sensitivity (at low strain rates) is not very different for the two polymers. The increased temperature sensitivity of PVC is, of course, related to its lower glass transition temperature as shown in Table 1.

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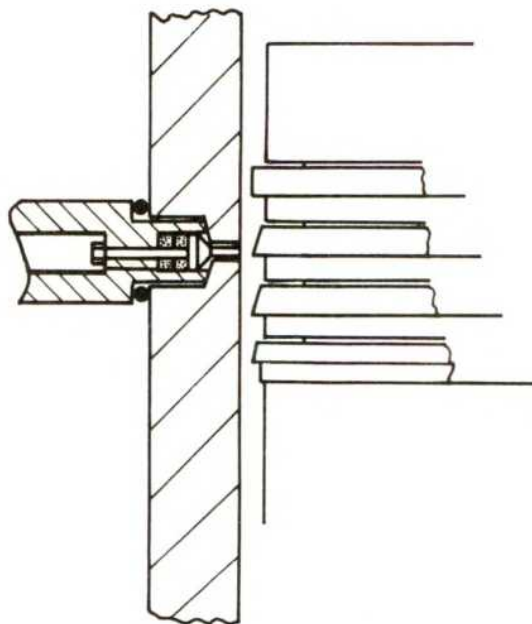
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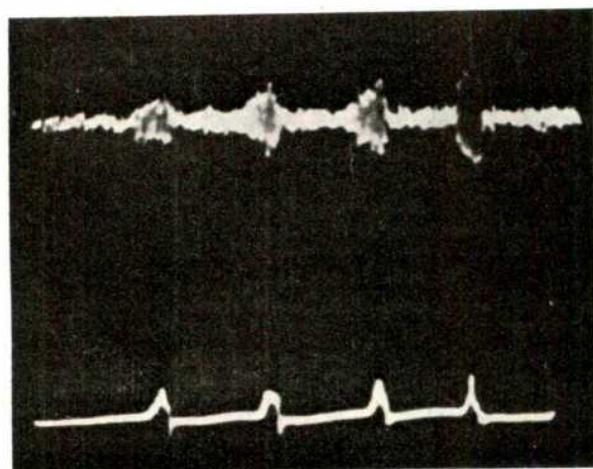
Hydrodynamic pressure under a piston ring

CASTLEMAN postulated¹ that the lubrication of piston rings might be hydrodynamic. Recently it has become possible to measure the oil film thickness directly, using a capacitance transducer². Oil film thicknesses of the order of $1 \mu\text{m}$ have been measured in a small diesel engine; theory predicts thicknesses of

**Fig. 1** The pressure gauge in the liner wall.

the order of $10 \mu\text{m}$. To investigate this discrepancy it was decided to measure the pressure in the oil film, since this provides an independent check on the theory.

A miniature pressure transducer was designed capable of operating in the conditions of a working engine. Essentially the transducer consisted of a cone-shaped plunger which transmitted the pressure from the cylinder face to a piezoelectric crystal (Fig. 1). The plunger was sealed at the front face of the cylinder by a flexible cement, the effective resolving power of the gauge being 0.025 cm . By mounting a capacitance transducer alongside the pressure gauge it was possible to obtain a synchronised oscilloscope display of pressure and film thickness.

**Fig. 2** Output from capacitance and pressure gauge on exhaust stroke.

Experiments have been carried out on a Petter AV1 diesel engine made available by Mr R. P. Langston of the Admiralty Oil Laboratory, Cobham. Figure 2 shows a result taken at 950 r.p.m. on the exhaust stroke. The upper trace is from the capacitance transducer; the presence of the rings is indicated by the out-of-balance signal. The lower trace is from the pressure gauge, the signal level being directly proportional to pressure. The first ring seen, on the left-hand side of the photograph, is the nominally parallel faced compression ring (running-in has

modified the profile to a slight taper), this is followed by two downward scraping taper-faced rings and finally a stepped-scraper ring. These traces show that the pressure distribution does not extend over the whole of the converging gap between the piston ring and the cylinder liner. This is contrary to the assumption of the elementary theory. The rings can be said to be operating in conditions of starved lubrication, presumably caused by the working of the ring-pack.

Details of the method of calibration together with a full account of the effect of engine conditions will be given elsewhere. Although the results do not conform completely with the details of simple theory, it is believed to be the first time that the pressures postulated by Castleman¹ have been observed directly.

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Field-desorption microscopy and the atom probe

WE have found unexpected and finely detailed field-desorption patterns, which demonstrate that the yields of metal ions from the surfaces of specimens in the field-ion atom probe are a function of the crystallography and sometimes also of the materials.

The field-ion atom probe¹, in which a field-ion microscope is combined with a time-of-flight mass spectrometer, has been shown to be a useful instrument for the chemical analysis on an atomic scale of metals and especially of alloys²⁻⁴. In an analysis, the field-ion specimen is manipulated to place an image feature over the entrance aperture of the mass spectrometer, which typically subtends only a few image points; a high voltage pulse is then applied to the specimen to desorb

a small amount of material. Ions desorbed from the selected feature pass through the aperture and their mass-to-charge ratios may be deduced from their flight times to a detector. The atomic resolution of the field-ion microscope in principle allows the feature to be chemically analysed atom by atom.

In practice, however, the ion yield is found to be a sensitive function of the position of the selected feature on the specimen surface. Brenner and McKinney⁵ have described systematic 'aiming errors' due to displacements of the trajectories of the field-evaporated metal ions from those of the ions of the image gas.

We have used a simple field-desorption microscope⁶ to investigate these phenomena directly. In this instrument an image is formed of the arrival positions of field-evaporated metal ions at the screen of a conventional field-ion microscope fitted with a micro-channel-plate image intensifier⁷. The image may be recorded directly using large aperture optics and a fast film (Kodak Tri-X or 2475 recording film), with up to 1,550 V applied to the channel plate; under these conditions the detection efficiency for single ions was estimated to be 20 to 50%. Alternatively, the channel plate gain and the lens apertures could be reduced to allow the image to be formed by the evaporation of many layers of the surface without saturation of the film. Proximity focused channel-plate intensifiers were found to be more suitable at very fast evaporation rates than magnetically focused intensifiers, which suffer from space-charge defocusing at the very high currents involved; this is likely also to apply to external image intensifiers.

When fractions of a monolayer of a tungsten surface were pulse evaporated to form the desorption image, micrographs similar to those presented by Walko⁸ and Panitz^{9,10} were obtained. Direct comparison of the field-ion and field-desorption images from the same surface demonstrates the general validity of Brenner and McKinney's conclusions concerning the directions of aiming errors, and allows their accurate measurement for different parts of the specimen surface.

When many planes of tungsten or other metal surfaces were evaporated to form the image, at reduced intensifier gain, very striking and unexpected intensity distributions were

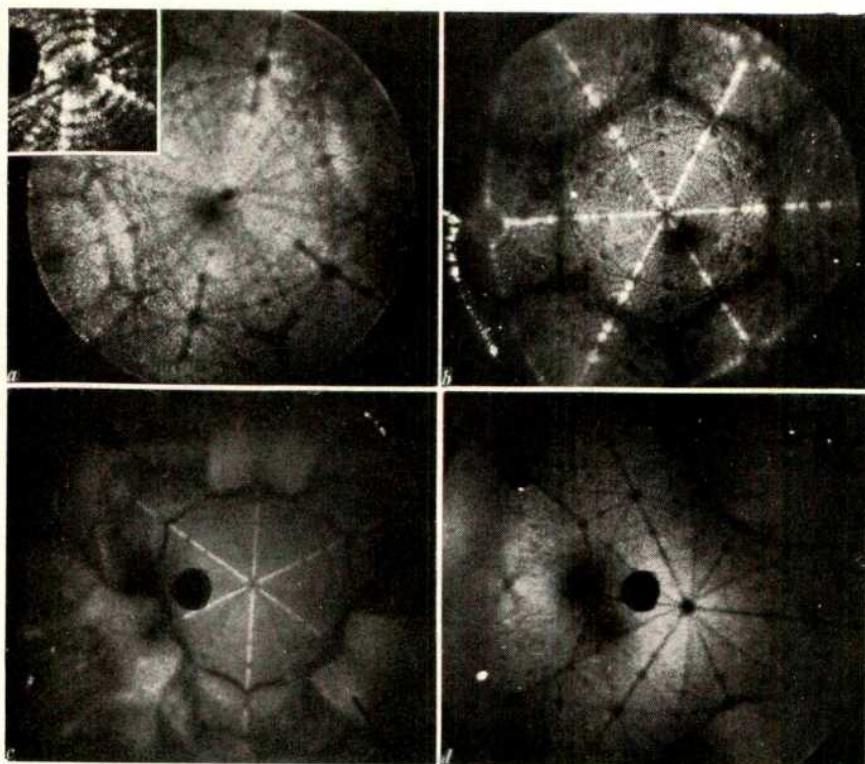


Fig. 1 Field-desorption micrographs at 78 K. *a*, Tungsten, <110> centred, evaporated by multiple 15-ns pulses; inset continuous evaporation, showing ring structure, zone decoration and radial bright lines towards the {211} planes. *b*, Rhodium, a typical f.c.c. pattern. *c*, Nickel. *d*, Aluminium in ultra-high vacuum. The f.c.c. patterns are all continuous evaporations and <111> centred.

obtained (Fig. 1 a-d). These became more well defined as more of the surface was evaporated, and were reproducible. Only minor variations were introduced by using evaporation pulses of 15 ns, 80 μ s or 500 μ s, or rapid continuous evaporation, or by superimposing many 15-ns pulses. As these patterns have been observed reproducibly under extremely diverse conditions using both proximity and magnetically focused image intensifiers, the possibility of instrumental artefacts (such as channel plate saturation¹¹) may be discounted. The patterns for the more refractory metals (such as W, Ir, Re, Mo) were largely independent of vacuum conditions in the range 2×10^{-10} torr to 5×10^{-8} torr at 78 K. In contrast, the pattern from the non-refractory aluminium was severely modified by as little as 5×10^{-9} torr of hydrogen.

The desorption patterns from b.c.c. metals (such as W, Ta, Mo, Fe) are as varied in character as the corresponding field-ion patterns. Those of the f.c.c. metals (Ir, Au, Rh, Pd), with the notable exception of aluminium, are all very similar, showing bright and dark line contrast with a faint background pattern of dark lines. A similar desorption pattern has been reported recently by Muller and Tsong¹⁰. Nickel is similar in the central region of a (111)-centred specimen, but shows a more complex pattern towards the edges, perhaps due to local mechanical deformation. Aluminium alone shows only dark lines in the desorption image. Dark centres to major planes are commonly seen, as are concentric ring structures centred on such planes (for example, {110} tungsten, {111} for Rh and Al); these structures persist even when hundreds of planes are evaporated to form the image.

We believe that the origins of these desorption patterns lie in short-range migration of atoms¹², induced by polarisation forces in the high electric field, along specific surface directions during the evaporation process; we do not think that differences in local magnification associated with differences in local curvature can alone lead to the observed detailed contrast, and in particular to the bright-line contrast, as originally suggested by Muller for platinum¹⁰. Coulomb repulsion between simultaneously evaporating ions is likely to contribute to the dark centres of major planes, which are more pronounced at high evaporation rates. Whatever the full explanation, we conclude that field-desorption microscopy will lead to a better understanding of the field-evaporation process and is an essential complement to atom-probe field-ion microscopy. This is particularly so for the analysis of very small precipitates and for analysis of segregants at grain boundaries, where we have observed both bright and dark contrast in the desorption micrograph. Provided that such effects are recognised, the atom probe remains the only instrument capable of accurate chemical analysis on an ultra-fine scale.

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Lead in urban street dust

LEAD pollution and its effects on health are matters of general concern^{1,2}. The potential danger to children is now receiving particular attention. Lead in various inorganic forms may be inhaled or ingested, and various sources of ingested lead (for example food, tapwater, paint) are recognised, and tolerable limits have been established. Recently, it was claimed² that for children in urban surroundings the dust of streets and playgrounds is a potentially significant source of lead, and that the lead in such dust can be over 1,000 p.p.m. (0.1%). This is a source of lead which is not immediately controllable, but we consider that its importance as a component of the lead intake of urban children has not been sufficiently assessed.

This investigation has two simple objectives: to determine the lead content of the dust and dirt likely to be encountered by children as a normal part of their environment in Greater Manchester, and to assess the importance of the dust as a component of the lead intake of urban children. Our results show an average lead concentration of 970 p.p.m., with little significant variation either with type of locality (main road, side street, playground, and so on), or with position within the urban area. Samples from nearby rural areas had average concentrations of 85 p.p.m. By comparison, typical values¹ for rural soils in Britain fall in the range 50 to 100 p.p.m., whereas the lead content of 'uncontaminated' soils is ~ 10 p.p.m. Our results for urban dust are similar to those obtained in other cities, for the few studies of which we are aware. In Birmingham, most values (R. Stephens, personal communication) were between 1,000 and 2,500 p.p.m. (average $\sim 1,700$ p.p.m.), the average for Rio de Janeiro³ was 700 p.p.m., and the averages for 77 cities in the USA (ref. 4) were between 1,500 and 2,400 p.p.m.

The 350 urban samples collected were of street dust, surface dirt or soil, from pavements, roadside gutters, children's playgrounds in parks, primary school playgrounds, gardens and waste land, over an area within ~ 10 km of the centre of Manchester, during the period December 1972 to March 1973. (In the south-east, the area sampled extended 15 km.) Twenty-five similar samples were also collected from a children's playground and a park in a rural area ~ 1 km outside the built-up zone, to the south-east of the area and ~ 15 km from the centre of Manchester. The samples were dried at 120° C and homogenised by grinding. Portions (~ 1 g) were boiled with 2 M nitric acid for 30 min. The lead content was determined by atomic absorption spectrophotometry and, for a randomly selected 10%

Fig. 1 Numerical distribution of lead concentration. Numbers above full arrows are percentages of samples below lead concentrations indicated.

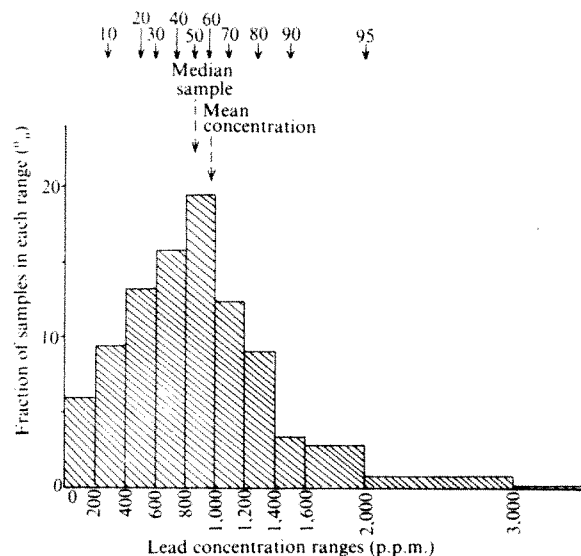


Table 1 Data subdivided according to type of locality (A–D, urban; E, rural)

Type	Definition	No. of samples	Mean Pb concentration (p.p.m.)	Standard deviation
A	Major roads/streets; moderate/heavy traffic 0800–2000	180	1,001	40
B	Minor roads/streets; light/moderate traffic 0800–2000	68	888	57
C	Streets with very light traffic only, and 'play streets'	53	933	186
D	School playgrounds; childrens' play areas in parks, gardens, waste land	49	1,014	206
E	Rural locations; childrens' playground and a 'country park'	25	85	40
A–D	All urban areas	350	970	46

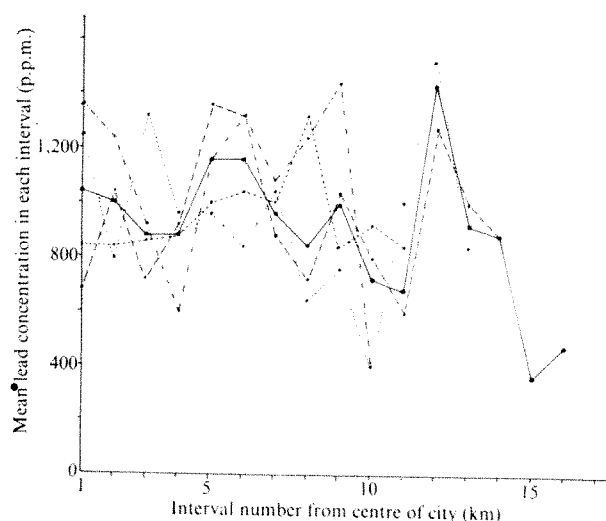
of the samples, by polarography. Agreement between the two methods was always better than $\pm 3\%$.

The average lead concentration in the urban samples was 970 p.p.m. (standard deviation 46); the numerical distribution is shown in Fig. 1. The highest value encountered was 10,200 p.p.m. and the lowest 90 p.p.m. But the higher values were unusual; only 10% of the samples had lead concentrations above 1,500 p.p.m. and only 5% above 2,000 p.p.m. The median value was 860 p.p.m. and the middle 50% of samples ranged from 550 to 1,200 p.p.m.

The urban samples were subdivided into four divisions, according to the type of locality (A–D, Table 1). There was no significant difference between the average lead concentrations in any of these subdivisions. The average lead concentration in the samples from the rural areas was 85 p.p.m., the type of locality being comparable with subdivision D.

We examined the geographical dependence of our analytical data in three ways. First, we subdivided the city into quadrants, and calculated the average lead concentrations for each quadrant. There was no significant difference between quadrants (Table 2).

In the second method we examined the data for a possible relationship between lead concentration and distance from the centre of the urban area. The distance of each sampling point from the centre (St Peter's Square) was calculated and the distances subdivided into 1-km intervals. The average lead concentration for the samples in each interval was calculated,

Fig. 2 Radial distribution of lead concentrations from the centre of Manchester. Quadrants NE, - - - -; SE, ---; SW,; NW, — · — · —; all, ———.

for each quadrant separately and for all samples. The resultant radial distributions (Fig. 2) fluctuated between about 600 and 1,400 p.p.m., with no significant general rise or fall with distance except for a sharp reduction at the distance corresponding to the edge of the urban area.

Third, we attempted to produce a density map of the area. For each 1-km square (National Grid), we compared the average value of the lead concentrations with the map of Greater Manchester. No general pattern emerged, and there was no apparent correlation with major roads or local centres of commercial and/or industrial activity.

It seems reasonable to assume that the bulk of the lead in the urban dust was at some time airborne. The principal sources of airborne lead¹ are probably vehicle emissions (> 90%), smoke from coal and refuse burning, and emissions from various metallurgical and industrial processes. The geographical distribution of lead contamination of the dust seems to support the view that much of the lead is airborne for periods of up to several weeks, and presumably more or less complete mixing of lead from the various sources must occur. As a result, a reasonably uniform dispersal takes place.

Table 2 Data subdivided according to quadrant of city (urban samples only)

Quadrant	No. of samples	Mean Pb concentration (p.p.m.)	Standard deviation
North-east (NE)	43	988	59
South-east (SE)	165	888	58
South-west (SW)	85	983	90
North-west (NW)	57	1,180	181

The importance of urban dust as a direct source of lead for humans must be assessed in relation to the intake from other sources, and the "provisional tolerable intake" recommended by the WHO. Considering only food, water and air as sources, ingested lead is estimated normally to contribute over 90% of the daily lead intake². Although less efficiently absorbed than inhaled lead⁶, ingested lead is by far the more important source^{5,7}. The recent *Survey of Lead in Food* (United Kingdom)⁸ estimates that for the average adult the intake of lead from food is $\sim 200 \mu\text{g d}^{-1}$, with a further $20 \mu\text{g}$ from liquids. A figure was not derived for children, but on the basis of the recommended caloric intake of food⁹, children (averaged over the ages 1 to 9) would reach 75% of the adult figure, $165 \mu\text{g d}^{-1}$.

A "tolerable intake" of ingested lead for adults was recently established (ref. 9 page 19, and ref. 10) at 3 mg per week (0.05 mg per kg body weight), equivalent to an average daily rate of $430 \mu\text{g}$, infants and children being specifically excluded. But the average weight of children (age 1–9) is $\sim 30\%$ of the adult average⁹, and on a weight ratio a tolerable daily intake would be $130 \mu\text{g}$. If children are more at risk than adults¹¹, this figure should presumably be reduced, and in any case will depend on age.

We conclude, therefore, that although adults on average ingest in food and drink only $\sim 50\%$ of their "tolerable intake", children ingest an amount close to or even above their tolerable level.

For adults, urban dust seems unlikely to contribute a significant quantity of lead to the daily intake. But for children even small quantities of dust would be significant. It seems to us that children who play in areas containing this type of dust are likely to ingest some of it, perhaps by casually eating sweets while playing in the street. We are not aware of any large scale determination of the amount of dust and dirt which is typically ingested by children. We have, however, made a small number of direct measurements and find that a child can transfer from 5–50 mg of dirt from his hands (dirty from 30 min of activity in a normal urban playground) to a typical 'sticky sweet' (5 g). An ingestion of $100 \mu\text{g}$ of lead (contained in 100 mg of average

dust) could result from the consumption, under these conditions, of from 2 to 20 sweets, which probably encompasses children's normal sweet-eating habits. Also, it is possible to envisage lead ingestions much greater than this—20 badly contaminated sweets could contain 1,000 μg of lead.

We conclude that children in urban surroundings, who may already be ingesting in food and drink an amount of lead approaching a tolerable limit, may considerably increase their daily lead intake by the accidental ingestion of dirt and dust from their surroundings in the course of their normal everyday activity. This source of lead does not at present seem to be recognised as a potential hazard. Thus, although upper limits for lead in food (generally 2 p.p.m.) or lead in paint for children's toys (0.5%) are recognised as being desirable, no tolerable upper limit for lead in urban dust has been suggested. We consider that this is an urgent and important problem.

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Lead levels in blood of residents near the M6–A38(M) interchange, Birmingham

LEAD levels in blood are generally found to be higher in city dwellers than in those living in the country^{1–6}. This difference has been linked to the increased background levels of lead, which result to a large extent from the emission of lead in motor car exhaust gases. In Birmingham, the opening of the M6–A38(M) interchange provided an opportunity to test this hypothesis by studying the effect of an increase in traffic density on the concentration of lead in the blood of those living close to the interchange.

Capillary blood samples were taken from volunteers living within 800 m of the interchange immediately before its opening in May 1972. Second blood samples were collected between October 1972 and February 1973, and a third collection was made in October 1973. Although a large number of samples were taken, only a relatively small number of persons contributed blood to each of the three series and it is the results from these people which are discussed here. All the estimations of blood lead were carried out at the same laboratory, using an atomic absorption technique. Because of some difficulties with skin contamination experienced during the collection of the first series of blood samples, venous blood was taken for the second and third analyses. No control group was studied.

Two groups were followed through the three series, the first consisting of 41 males and the second of 58 females. The mean

blood lead concentrations in the three series were, for the male group, 14.41, 18.95 and 23.73 μg per 100 ml and for the female group, 10.93, 14.93 and 19.21 μg per 100 ml. For both groups the differences between the first and second, second and third and first and third means are statistically significant as judged by paired *t* test analysis (Table 1).

Table 1 Statistical analysis of mean lead concentrations in the blood of two groups of residents living near the M6–A38(M) interchange

		Series		
		1:2	2:3	1:3
Males	Mean difference	4.54	4.78	9.32
	Standard error	1.72	1.55	1.46
	<i>P</i>	<0.05	<0.01	<0.001
Females	Mean difference	4.00	4.28	8.28
	Standard error	0.94	0.87	0.95
	<i>P</i>	<0.001	<0.001	<0.001

The M6 opened on May 24, 1972. In April 1972, 361,040 cars a week used the road later incorporated into the interchange. By March 1973, 748,639 cars a week were using the whole interchange and by March 1974 the weekly figure was 900,451.

When interpreting these results due consideration must be given to the fact that the method of blood sampling was changed after the first series and that the samples in each of the three series were not all taken at the same time of year. During the course of the study, however, capillary and venous samples were taken on the same occasion from a total of 96 different individuals in the main study (47 males and 49 females) and it was found that the blood lead concentration in the capillary samples tended to be higher than in the venous samples. Thus any bias introduced into the study by changing the sampling method would seem to be against showing a rise in the blood lead concentration. Similarly, it has been shown that the blood lead concentration is higher in March–July than during the rest of the year⁷ so that in taking the second and third samples during October–February, and not in May when the first sample was collected, a bias is again introduced which is against showing an upward trend. Thus, despite the defects in experimental design which undoubtedly mar the study, the results suggest an increase in the mean blood lead concentration of the two groups of residents over a period when the volume of traffic on the motorway was increasing. The blood lead concentrations are, however, higher than might have been predicted from the atmospheric concentrations using the model of Knelson *et al.*⁸ The concentration of lead in the atmosphere in the residential areas near the motorways is approximately 1 $\mu\text{g m}^{-3}$ although values greater than this are found at sites closer to the road⁹. Knelson and Bridbord¹⁰ have suggested that continued exposure to atmospheric lead levels of the order of 1–3 $\mu\text{g m}^{-3}$ will produce increases in the blood lead concentration, and their theoretical considerations have received some support from experiments on human subjects exposed to low atmospheric lead concentrations produced under controlled conditions¹¹. In these considerations, however, the total amount of lead in the atmosphere is of less importance than its particle size. The model proposed by Knelson⁸ assumes a constant pulmonary absorption rate of 37%. Now if the lead aerosol particles around the interchange are small enough to enable the aerosol to assume the properties of a gas, then maximum lung penetration will be achieved and pulmonary absorption may well be considerably higher than 37%. A preliminary study¹², which has shown that the atmospheric lead levels inside one house near the interchange are almost identical with those outside and follow the same diurnal fluctuation, is consistent with the notion that the aerosol is, in fact, behaving as a gas.

A number of workers^{13–16} have found the mass median diameter of airborne lead particles to be of the order of 0.10–0.90 μm although electron micrographs have shown that

the lead particles may form large aggregates with other material in the atmosphere¹⁷. Whether this aggregation takes place during emission from the tail pipe or during sampling is, however, unresolved. Clearly it would be of considerable interest to have more information about the physical and chemical characteristics which might affect pulmonary absorption of the particles of lead in the atmosphere around the interchange. It should also be borne in mind that factors other than the inhalation of lead from the atmosphere might be contributing to the increase in blood lead—for example, the ingestion of lead-contaminated dust.

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Interactive effects of unpleasant light and unpleasant sound

IN tests of colour preference, rhesus monkeys have been found to have a strong aversion to light at the red end of the spectrum^{1,2}. No comparable reaction to colours has been described in healthy human beings although in patients who are suffering from cerebellar or spinal disorders colours may assume a new potency. Thus it has been claimed that in cerebellar patients who exhibit the so-called 'sensorimotor induction syndrome', red light not only exacerbates the motor disorder but disrupts thought processes and leads to acute subjective distress, whereas blue-green light alleviates the symptoms^{3,4}. Furthermore, Halpern and Feinmessenger⁵ found that in such patients red light, besides causing discomfort in itself, increases the sensitivity to noxious auditory stimulation: in their experiments the 'threshold of acoustic discomfort' was consistently lowered when the patients wore red filters in front of their eyes. So we have investigated in monkeys whether the aversion to red light is increased when the preference tests are conducted in the presence of unpleasant background noise.

The technique for measuring the monkey's reactions to red light was similar to that previously described². The monkey sat in a small dark chamber with a screen (40 cm × 40 cm) at the far end, on to which red or white light could be projected. The red light (Kodak Wratten filter 25) and the white light were matched in subjective brightness at 1.5 log foot lamberts. The monkey controlled the presentation of the two stimuli by pressing a button. Successive presses

on the button produced the red and the white light in strict alternation, either stimulus staying on for as long as the button was held down. Each test lasted for 400 s of total exposure, during which time the monkeys typically alternated between the two stimuli about 100 times. The relative preference for the red light was measured as the ratio of the time spent with the red light to the total time spent with either red or white light over a defined interval.

The effect of background noise on the colour preference was investigated by comparing the monkey's performance when the chamber was quiet with that when 'white noise' was played through an overhead loudspeaker during the test. The sound pressures in the 'quiet' and the 'noisy' conditions were 59 db and 80 db respectively. For comparisons to be made within a single test, each test was subdivided into eight 50-s bouts, four 'noisy' and four 'quiet' presented alternately. Each monkey was given twenty

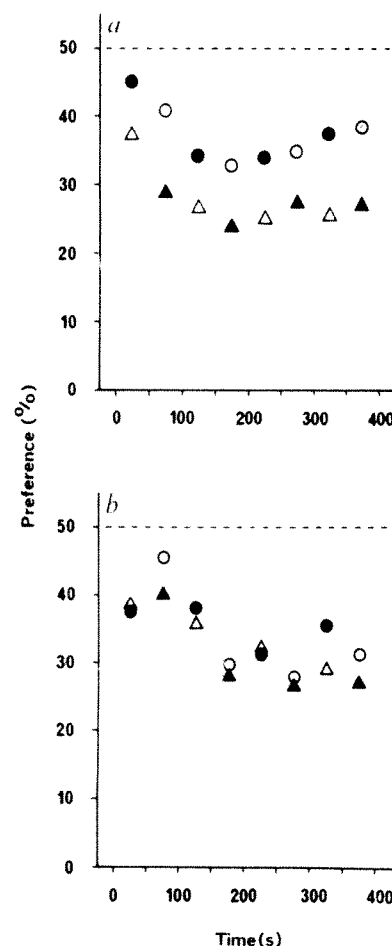


Fig. 1 Preference for red over white light in each successive 50-s period. *a*, Monkeys 1 and 2; *b*, monkeys 3 and 4. ○, ●, 'Quiet' condition; △, ▲, 'noisy' condition. ○, △, Tests which began with noise; ●, ▲, tests which began with quiet. Each point represents the mean of the two monkeys' mean scores over ten tests.

tests, ten of which started with 'noise' and ten with 'quiet'.

Four young adult male monkeys (*Macaca mulatta*) served as subjects. Monkeys are not all equally reactive to white noise and these four were selected as being two who found noise extremely aversive and two who found it relatively tolerable. This assessment was made by running preliminary tests in which the monkeys were given the opportunity to choose between noise and quiet in a simple (noise+light)/(quiet+light) preference test⁶. In five tests, each lasting 400 s, monkeys 1 and 2 preferred the quiet condition 85% and 83% of the time, while monkeys 3 and 4 preferred it 65% and 68% of the time.

The results of the main experiment are shown in Fig. 1.

the two subgroups of monkeys being treated separately. For monkeys 1 and 2 the background noise increased the aversion to red light; for each of these monkeys the effect was significant beyond the 0.001 level (Wilcoxon test, based on the performance within each of the twenty tests). For monkeys 3 and 4, however, the noise had little if any influence; although each of these monkeys showed a marginally greater aversion to red light in the noisy condition, the effect was not significant. The implication is that background noise increases the aversion to red light if, and only if, the monkeys find the noise itself markedly aversive.

Although Halpern and Feinmesser studied the effect of red light on the aversion to noise, whereas we did the reverse in our experiment, there is an obvious parallel between the results of the two studies. The difference in experimental design is in fact more apparent than real, since, whichever way round the experiment is done, the most one can conclude is that a combination of red light and noise is unduly aversive. The experiments do not allow one to distinguish whether it is red light which increases the unpleasantness of noise or noise which increases the unpleasantness of red light; indeed, this is probably a false antithesis, the truth being that the effects of the two stimuli mutually potentiate each other.

Leaving aside the puzzle of why red light should be aversive to either monkeys or cerebellar patients, one may speculate on the broader implications of these findings. Whether it is a general rule that unpleasant stimulation in one modality potentiates the effect of unpleasant stimulation in another remains unknown; but a rule of this sort would be given a certain plausibility by Stevens' 'power law' for subjective sensation⁷. Stevens showed that when people are given electric shocks their subjective discomfort is an accelerating function of the magnitude of the physical stimulus (thus the discomfort resulting from a 40 V shock is more than twice that from a 20 V shock). Suppose, purely for illustrative purposes, that red light alone is equivalent to X units of shock, and noise alone is equivalent to Y units of shock, then the discomfort due to a combination of red light and noise may be expected to be greater than the sum of the discomfort caused by the component stimuli, since $(X+Y)^n > (X^n + Y^n)$, if the sensory exponent, n , is greater than one.

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Insect hormones as tsetse abortifacients

Female tsetse flies produce only one offspring at a time, the larva being retained within the uterus where it is nurtured from the female's milk gland¹. The low reproductive potential resulting from this curious form of viviparity is a feature that can perhaps be exploited as a vulnerable link in the life cycle. My experiments show it is possible to disrupt the normal 9 d pregnancy cycle² by using insect hormones to induce abortion.

Glossina morsitans morsitans Westw. was used in the experiments reported here. Females were transferred from

mass culture to individual containers³ shortly before the first larviposition. Flies were offered a blood meal on rabbit ears 6 d a week and feeding records were kept for each fly. The hormones were applied during the second pregnancy cycle; all stages of pregnancy were represented in approximately equal proportions for each dose of hormone applied. Crystals of plant-derived ecdysterone were dissolved in 96% ethanol and diluted with distilled water to 10%; 0.5 μ l was injected into the thoracic tergum by a glass capillary. The juvenile hormone analogue isopropyl (2E,4E)-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate (ZR515) was applied to the abdomen in 5 μ l acetone. The flies were held between finger and thumb and were not anaesthetised. Each fly container was examined daily for aborted progeny.

As is seen in Table 1, the effective dose of ecdysterone required to elicit a 50% rate of abortion was between 1.0 and 10.0 μ g. Most abortions occurred during the on-going pregnancy, but a few females aborted again in the next pregnancy. A few aborted only during the next pregnancy. Observations on 45 females kept beyond the second post-injection pregnancy suggest that later pregnancies and female longevity were not affected.

Topical application of the juvenile hormone analogue (JHA) caused a 50% rate of abortion at a dose of approximately 10 μ g. As with ecdysterone, the effect was most pronounced during the on-going cycle (Table 1).

Both hormones induced abortions in the egg stage as well as in all three larval instars. Although the hormones were applied to individuals representing all stages of pregnancy,

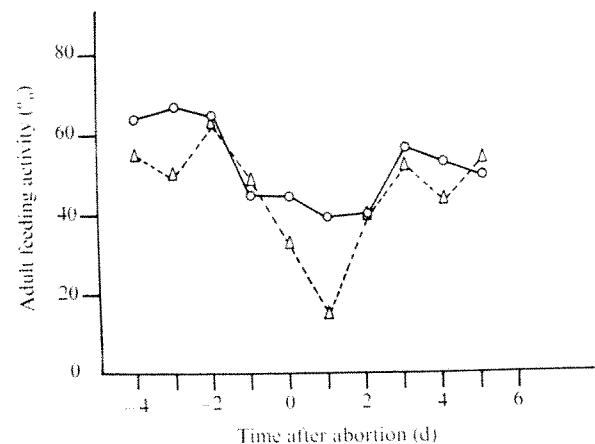


Fig. 1 Incidence of daily feeding activity in tsetse females that aborted following either an ecdysterone injection (○) or a topical application of a juvenile hormone analogue (△). Feeding activity is expressed as the percentage of females that took a blood meal during a 15 min exposure to rabbit ears. Each curve is based on the record of 38-40 flies.

the effect was most frequently manifested in the abortion of 2nd or 3rd instar larvae. The aborted 3rd instars were not fully grown; melanisation of the cuticle² was frequently incomplete and they failed to pupariate. In the case of ecdysterone, 72% of the abortions ($n=43$) were of 3rd instars and 21% of 2nd instars. With JHA 38% of the abortions ($n=42$) were of 3rd instars and 43% were of 2nd instars. The time between hormone application and abortion was much shorter with the JHA: 42% occurred within 24 h and several aborted within 2 h. For abortions occurring during the 1st pregnancy cycle, the latent period was 2.4 ± 0.5 d (mean \pm s.e., $n=36$). In the ecdysterone-treated flies only 7% aborted within 24 h and the mean latent period was 4.2 ± 0.5 d ($n=29$).

All the abortions depressed feeding activity below normal for several days (Fig. 1). For the JHA a pronounced

Table 1 Incidence of abortion in *Glossina morsitans morsitans* Westw. resulting from injection of ecdysterone or topical application of a juvenile hormone analogue

Substance	Treatment	Dosage (μg)	No. of females	Distribution of no. of abortions during two subsequent pregnancies			Females aborting (%)
				1st cycle only	2nd cycle only	1st and 2nd cycle	
10% Ethanol	—	—	23	0	2	1	13.4
Ecdysterone	0.01	—	24	0	4	0	16.6
Ecdysterone	0.1	—	25	3	2	1	24.0
Ecdysterone	1.0	—	23	5	1	1	30.4
Ecdysterone	10.0	—	26	17	1	3	80.8
Acetone	—	—	29	2	0	0	6.9
JHA (ZR515)	0.1	—	25	1	0	1	8.0
JHA (ZR515)	1.0	—	25	3	2	0	20.0
JHA (ZR515)	10.0	—	25	9	1	2	48.0
JHA (ZR515)	50.0	—	36	13	4	4	79.0

decrease in activity occurred on the day following abortion. In spite of a decline in feeding around the time of abortion, normal larvae were produced in 10.4 ± 1.2 d (mean \pm s.e., $n=25$) after an ecdysterone-induced abortion, and in 9.2 ± 0.6 d ($n=23$) after a JHA-induced abortion.

Effective tsetse abortifacients could have several possible modes of action; first, direct influence on the act of parturition; second, 'turning off' the milk gland; third, disruption of *in utero* larval morphogenesis; or fourth, desynchronisation of the (presumed) female neuroendocrine integration of normal pregnancy².

Larvae within the uterus of a starved female can survive several days before the onset of starvation-induced abortion⁴; therefore it seems unlikely that the rapid abortion initiated by JHA could be the result of effects on the milk gland. As feeding activity in hormone-treated females (Fig. 1) was within the expected range² before abortion, malnutrition is an unlikely cause for the expulsion of immature larvae. Moreover, large milk glands were observed in numerous females that aborted.

Pharmacological agents that act on neuromuscular processes involved in parturition could also potentially act as abortifacients. Physostigmine, a reversible anticholinesterase, was moderately effective as a fast-acting abortifacient. Injection of $0.5 \mu\text{l}$ of a 0.1 mM solution of physostigmine (B.D.H. Ltd) in 10% ethanol caused 4 out of 19 females to abort within 15–30 min. All females that did not abort within 30 min successfully completed their pregnancy. Injection of a higher dosage ($0.5 \mu\text{l}$ of a 1.0 mM solution) caused 100% female mortality. Prostaglandin E_1 and prostaglandin F_2 α -tromethamine salt (Upjohn Co.) injected in dosages of $10 \mu\text{g}$ in $1 \mu\text{l}$ 10% ethanol were ineffective.

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Behavioural role of individual components of a multichemical attractant system in the Oriental fruit moth

In most moths, location of the female for mating is mediated by pheromones¹, which are operationally defined as attractants, although their ability to elicit upwind orientation has been demonstrated in only a few species². Intrinsically unattractive chemicals which modify this process by increasing or decreasing trap catch have been termed synergists and inhibitors¹, respectively, although their behavioural and neurophysiological roles have remained unresolved. In the Oriental fruit moth, *Grapholitha molesta* (Busck), *cis*-8-dodecenyl acetate (*c*8-12:Ac) is a primary pheromone component³ and recently the requirement for attractancy of an isomeric mixture containing about 8% *trans* (*t*8-12:Ac) was demonstrated^{4,5}. Additionally, male trap catch has been reported to be enhanced about two-fold by the simultaneous release of dodecyl alcohol (12:OH) (ref. 5). Specific behavioural functions, however, had not been ascribed to individual attractant components or combinations.

Table 1 Male behaviour within 3 m of a white sticky trap baited with attractant on a rubber septum dispenser

Stimulus	No. of males observed	Percentage captured*	Mean flight time† (s)
100 μg <i>c</i> 8-12:Ac (6.8% <i>trans</i>)	39	56	19.8
100 μg <i>c</i> 8-12:Ac (6.8% <i>trans</i>) + 300 μg 12:OH	40	93	12.5

*Compared with a 2×2 test of independence using the *G* statistic with Yates' correction: $P < 0.01$.

†Means compared with the *t* test: $P < 0.05$. Measurements were initiated on the arrival of males within 3 m of the trap's edge and terminated when males either left this area or were ensnared on a trap's lower sticky surface.

Field studies in Geneva of wild male behaviour near baited traps showed that 100 μg *c*8-12:Ac alone failed to lure males to the trap vicinity, and indeed no males were observed orienting toward the traps within about 15 m. The combination of *c*8-12:Ac with 6.8% of the *trans* isomer did lure males to the trap (Table 1), and the simultaneous release of 12:OH effected a increase in the number of males actually captured on the sticky trap surface. In terms of trap catch, the attractant 'synergist' (12:OH) seems to increase the frequency of male landing.

Detailed field analyses of the role of 12:OH were carried out using an attractant dispenser placed in the centre of a circular table top of radius 60 cm. Previous field trials had revealed that 10 μg *c*8-12:Ac with 6.8% *trans* elicited the closest mean approach to the dispensers, at doses between 1 and 3,000 μg . When various quantities of 12:OH were present along with 10 μg of the attractant, the presence of 12:OH resulted in significant increases in the frequencies of

Table 2 Behavioural effects on 12:OH when presented simultaneously with 10 µg of c8-12:Ac (6.8% *trans*) on a rubber septum dispenser

12:OH (µg)	No. of males observed	Percentage landing on table top*	Percentage fanning while walking on table top*	Percentage hair-pencil display on table top*	Mean closest approach† to attractant dispenser (cm) ± s.e.
0	37	68	27	3	47.5 ± 2.5
1	40	88‡	73¶	8	33.6§ ± 3.2
3	39	77	36	23‡	36.7‡ ± 3.9
10	33	94¶	83¶	42¶	19.9§ ± 3.9
30	40	80	45	15	35.3¶ ± 3.6
100	30	83	43	30‡	28.5§ ± 4.3

*Percentages in the same column compared with the treatment lacking 12:OH with a 2 × 2 test of independence using the *G* statistic with Yates' correction.

†Means compared with the treatment lacking 12:OH according to the *t* test. Males flying within 0.5 m of the table top edge but not landing were scored as approaching within 60 cm. The remaining approaches were by males walking on the table top.

‡ *P* < 0.05

¶ *P* < 0.01

§ *P* < 0.001

landings, wing fanning while walking, approach to the chemical stimulus and extrusion of hair-pencils⁶ at the abdominal tip (Table 2). This is the first example of a particular chemical component evoking hair-pencil display.

Although to date only c8-12:Ac has been confirmed from female *G. molesta*, this species seems to utilise a multicomponent communication system. Both c8- and t8-12:Ac are requisites for attraction but this combination seems to elicit relatively little fanning and almost no extrusion of hair-pencils close to the attractant source. The main effect of 12:OH, previously considered an attractant synergist, is apparently to elicit a repertoire of precopulatory behaviour, which is likely to increase the catch in a trap.

The finding of disparate communicative functions for chemicals thought to be attractants and modifiers has implications for control programmes based on the disruption technique, in which the pheromone permeates the atmosphere so that either the males fail to locate the females (confusion) or habituation of mating responses⁷ occurs. Successful implementation of this procedure may ultimately depend on an intimate understanding of the precise communicative role of individual pheromonal components, since manipulation of attraction alone may not obviate mating.

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Coral diseases in Bermuda

WE report here diseases of reef corals that seem to be associated with bacterial infection. On reefs around Bermuda, where our field work was carried out in the summer of 1973, the most commonly affected species are the brain corals *Diploria labyrinthiformis* and *D. strigosa* (Fig. 1). We believe that this is the first unequivocal report of a coral disease and our observations suggest that diseases may be important agents of coral death. (There are some indications that others have seen diseased corals^{1,2}.)

The distribution of diseased colonies is difficult to evaluate because only 0.5–1% of the *Diploria* colonies are affected at a given time. Diseased colonies, however, occur throughout the reefs. Their distribution seems unrelated to inshore

pollution sources and to areas of greater or lesser circulation.

On the reefs the *Diploria* disease appears as a dark brown line separating a dead from a live portion of a colony. The coral is apparently healthy, with all its zooxanthellae up to the disease line (Fig. 2), while on the other side of the line no tissue remains. The disease consumes tissue at a rate of a few centimetres per month. The exposed corallum behind the line of infection is soon covered with filamentous algae and diatoms.

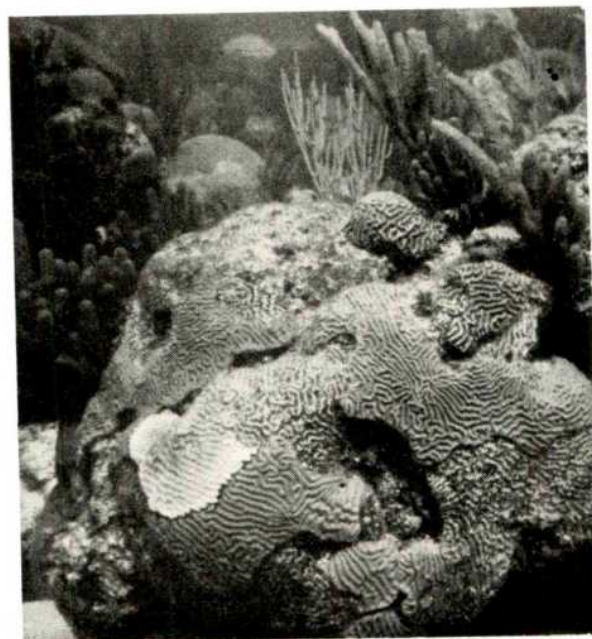


Fig. 1 Infected colony of the brain coral *Diploria strigosa* in 5 m of water at North Rock, Bermuda. The infected area, with black disease line and exposed white corallum, is at the lower left.

The disease seems to initiate at the margins of living colonies, or at irregularities such as areas of boring bivalves or barnacles, and to spread outwards from such points. We have seen no evidence for the initiation of the disease in the middle of healthy tissue. The disease does not always kill an entire colony, but rather dies out itself in time, leaving colonies with dead patches and living areas, the latter raised up by their continued growth.

Microscopic examination showed the disease to be a dense mat of bacterial filaments in which diatoms, ciliates and released zooxanthellae are embedded. The principal organism is the gliding bacterium *Beggiatoa*, a heterotroph requiring a source of sulphide such as H₂S to initiate its catabolic activities³.

By exposing healthy *Diploria* colonies to reducing conditions in aquaria, we have been able to foster *Beggiatoa*

infestations. In our experiments, reducing conditions over the coral surface were exploited by the sulphate-reducing anaerobic bacterium *Desulfovibrio*, which produced copious amounts of H_2S gas. Then *Beggiatoa* colonised the air- H_2S interface and attacked the coral tissue.

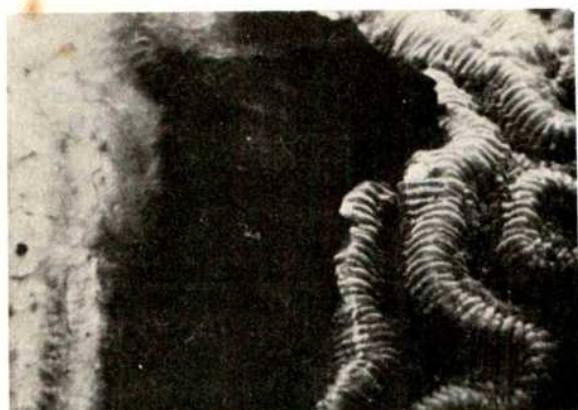


Fig. 2 Close-up view of the disease line on *D. strigosa*. Healthy tissue on right. The disease line is approximately 15 mm wide.

Such artificial infestation has not produced the localised dark line disease, but we find *Desulfovibrio* and *Beggiatoa* in the natural as well as in the artificial infections. The involvement of these microorganisms in both types of disease may be related only to decay (that is, the end stage of the disease). The close proximity of the *Beggiatoa* mat to healthy tissue in the natural disease, however, suggests its involvement at least in the spreading, and possibly in the initiation of the disease.

We have also occasionally observed the dark line disease on *Montastrea annularis*, and a disease net, similar to our artificially induced disease, on *Porites astreoides*. It seems likely that such diseases attack other corals in other reef areas, although because of the short life span of the disease (weeks to months) the numbers of diseased colonies will always be small. The large number of coral colonies on the reefs of Bermuda with dead patches, many of which could be disease-related, indicate that disease may be an important factor in coral ecology.

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in association with callus cultures of non-legumes as well as with cultures of legume species which do not form nodules with the cowpea bacteria.

Three rhizobial strains were tested for contamination by non-symbiotic dinitrogen-fixing bacteria and maintained on a medium containing K_2HPO_4 0.5 g; $MgSO_4 \cdot 7H_2O$ 0.2 g; NaCl 0.1 g; yeast extract 0.5 g; $CaCO_3$ 0.5 g; mannitol 10 g l^{-1} water. Tissue cultures of brome grass (*Bromus inermis* Leyss.), wheat (*Triticum monococcum* L.), rapeseed (*Brassica napus* L.), sweet clover (*Melilotus alba* Desr.) and *Vicia hajastana* (Prairie Regional Laboratory⁵) were routinely examined by light microscopy, and subcultured on nutrient agar and in nitrogen-free media³. These tests confirmed the absence of microbial contaminants. Nitrogenase activity was established essentially as described for the soybean-*R. japonicum* combination⁶. Plant cells from suspension cultures were placed on a membrane lying on top of 50 ml low-nitrogen nutrient agar. After callus growth was established (7-20 d) the surface was infected with a small volume of *Rhizobium*. After a further 7-14 d, the vessel was sealed and endogenous ethylene production measured by gas chromatography after 1 h. Acetylene was injected, and after an hour another sample of the gas phase removed for analysis. The reduction of acetylene to ethylene is a positive indication of nitrogenase activity⁷. The plant cells were removed from the membrane for microscopic examination and dry weight determination.

Rhizobium sp. 'cowpea' 32H1 formed nitrogenase (C_2H_2) in association with plant cells of five field pea varieties, two soybean varieties, sweet clover and *Vicia hajastana*, as well as the non-legumes rapeseed, wheat and brome grass (Table 1). The possibility of infection by non-symbiotic dinitrogen fixing bacteria was excluded by subculturing positive callus-rhizobial associations on to yeast extract-mannitol agar, glucose-peptone agar and *Azotobacter* media³. There was no growth on glucose-peptone or nitrogen-free nutrient, and colonies observed on yeast extract-mannitol agar were microscopically indistinguishable from cowpea rhizobia. The dinitrogen fixing association described is aerobic^{6,8} and it is unlikely that it is due to contamination by *Klebsiella*, which usually only fixes anaerobically. The nutrient used⁶ has a low pH (5.5), and is unlikely to permit

Table 1 Acetylene reduction activity of associations of *Rhizobium* sp. 32H1 and various plant callus cultures

	Plant cells + <i>R. sp.</i> 32H1	Specific activity (nmol $C_2H_4\text{ h}^{-1}\text{ g}^{-1}$ dry weight) uninfected plant cells
Pea var. 'Century'	530	1.7
'Trapper'	1,250	8.8
MP766	109	2.5
MP7608	251	2.1
EMD	413	5.7
Soybean var. 'Acme'	353	—
'Mandarin'	7.3	—
<i>Vicia hajastana</i>	335	0.9
Sweet clover	108	3.6
Rapeseed	131	5.7
Wheat	83	0.6
Brome grass	323	0.2

Nitrogenase activity was determined by the reduction of C_2H_2 to C_2H_4 , followed by determination of the dry weight of callus. Each value is the mean of five samples. Rhizobial strains were obtained from Dr J. C. Burton, the Nitragin Company, 210 West Custer Ave, Milwaukee, Wisconsin 53209, USA. Strain 32H1 and 32Z3 were isolated from *Crotalaria* and strain 21A was isolated from *Chamaerista fasciculata*. Single colony isolates were prepared from dilution plates. All strains are motile Gram-negative rods, produced effective nodules on cowpea plant grew well on yeast extract-mannitol agar, and formed uniform pale colonies on yeast-mannitol-congo red agar. The three strains form a slight 'serum zone' in litmus milk after 10-14 d incubation². There was no growth on glucose-peptone agar, sporulation agar or severe standard nitrogen-free media³. Absence of *Klebsiella* was indicated by the lack of growth, acid or gas formation in anaerobic semi-solid peptone-carbohydrate media⁴.

Nitrogen fixation by a *Rhizobium* sp. in association with non-leguminous plant cell cultures

THE genus *Rhizobium* is characterised by its ability to elicit nodule formation and fix dinitrogen in roots of legumes and its species or groups are classified according to their legume host range. There is only one non-legume association reported, that of *Trema aspera* with a 'cowpea-type' strain of *Rhizobium*¹. Dinitrogen fixation by *Rhizobium* in the absence of a host plant has not yet been demonstrated. I report the production of nitrogenase (C_2H_2) activity in *Rhizobium* sp. 'cowpea strain' growing

dinitrogen fixation by *Azotobacter*, which usually prefer an almost neutral pH

Examination by light or electron microscopy showed that rhizobia grew on the surface of the callus and in cracks and intercellular spaces. Where bacteria were found in plant cells, destructive invasion had occurred. Moreover, the few such infected plant cells could not account for the observed acetylene reduction. In no case was a plant cell containing rhizobia observed which looked like a normal nodule cell. The bacteria always appear as rods rather than bacteroids, and no membrane surrounding the bacteria could be detected. The *Rhizobium*-plant callus association differs from the nodule system in its morphology and its reaction to plant growth regulators^{6,8}. A nodule-like infection process does not seem to be necessary to establish nitrogenase activity in the tissue culture system.

After the membranes were lifted to remove the callus for examination, a copious growth of rhizobia was often seen on the agar. The vessels were resealed, and acetylene again added. After an hour, ethylene production was measured, and it was found that 5–25% of the nitrogenase activity of the original systems could be accounted for by these bacterial cells not in direct contact with plant callus. The nitrogenase activity continues for 24 h, but does not persist when these rhizobia are transferred to nitrogen-free media.

Table 2 Acetylene reduction activity of associations of three strains of 'cowpea' *Rhizobium* and various callus cultures

Rhizobia sp Plant cell line	Specific activity (nmol C ₂ H ₄ per h per g dry weight)		
	32H1	32Z3	21A6
Soybean var 'Acme'	105	56	0
<i>Vicia hajastana</i>	178	26.5	23.9
Sweet clover	114	9.0	7.0
Rapeseed	205	19.0	20.6

Nitrogenase activity determined as in Table 1

The ability to form nitrogenase in the presence of non-host plant cells was also found in three cowpea strains (Table 2). Nitrogenase activity was, however, not obtained from *R. japonicum* or *R. leguminosarum* in the presence of non-legume tissue cultures.

Phillips⁹ has reported nitrogenase activity in an association of soybean cells and cowpea strain 32H1. This was not surprising, as *R. japonicum* and the cowpea groups are closely related, both belonging to the 'slow growing' rhizobia, and cross inoculation between soybean and cowpea has been found². Clover, pea and *Vicia* are nodulated by the 'fast growing' group of *Rhizobium* and nodulation of intact plants by cowpea *Rhizobium* is unknown².

The genes coding for nitrogenase are found in the bacteria^{10,11}. What elicits the expression of nitrogenase activity is unknown but the diffusible factor(s) involved are obviously not limited to legumes. The demonstration of nitrogenase in the presence of legume, rapeseed, wheat and brome grass cells suggests that some common plant product may be involved. It is likely therefore that in symbiotic dinitrogen fixation the species barriers are at the stages of infection and nodule formation, and not in the expression of nitrogenase.

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Nitrogen fixation by *Rhizobium* associated with tobacco and cowpea cell cultures

THE maxim that nitrogen fixation by the root nodule bacteria, *Rhizobium*, is restricted to a formal symbiotic association with specific legumes has recently been challenged. Trinick¹ showed that nodules formed on the non-legume *Trema canabina* (previously identified as *T. aspera*, M. J. Trinick, personal communication) by a strain of *Rhizobium* which nodulated *Vigna unguiculata* (cowpea), possess nitrogenase activity and fix atmospheric nitrogen. Soybean tissue cultures inoculated with *R. japonicum*^{2–4}, or with cowpea strains of rhizobia³, also possess apparently functional nitrogenase as determined by the acetylene reduction assay⁵. Several attempts have failed to demonstrate nitrogenase activity in cultured rhizobia⁶, including cowpea strains⁷.

We report nitrogenase-mediated acetylene (C₂H₂) reduction, and ¹⁵N incorporation, in associations of a strain of cowpea rhizobia (32H1) with cell cultures of a natural host, *V. unguiculata*, but more notably with a non-legume, *Nicotiana tabacum*. Furthermore, this strain of rhizobia reduced C₂H₂ when cultured adjacent to, but not in contact with, tobacco callus.

Cell cultures, derived from stem explants of *N. tabacum* cv Wisconsin 38 and *V. unguiculata* cv Poona, were maintained as 30-ml suspensions in Schenk and Hildebrandt⁸ (SH) medium with 2,4-dichlorophenoxyacetic acid (9 μM) and kinetin (0.2 μM) in 250 ml flasks on a gyratory shaker (100 r.p.m.) at 29°C in the dark. A culture of strain 32H1 (J. C. Burton, Nitragin Co., Milwaukee, Wisconsin⁹) was checked for purity by two successive isolations of single colonies on yeast-mannitol agar. Actively growing plant cell suspensions were inoculated with 2 × 10⁸ bacteria (1 ml), shaken for 48 h, centrifuged, and washed in cell culture medium. Aliquots (0.8 g fresh weight) of the pellet were spread on filter paper, drained of excess liquid and the cells and filter paper incubated on the surface

Table 1 Acetylene-dependent ethylene production* by 32H1-plant cell associations

	<i>N. tabacum</i> (nmol C ₂ H ₄ per g dry weight per hour)	<i>V. unguiculata</i> (nmol C ₂ H ₄ per g dry weight per hour)
GLN medium†	106 ± 21	7 ± 2
RN medium	118 ± 8	6 ± 2

*Cultures were sealed with serum stoppers and assayed after 2 h for endogenous ethylene (C₂H₄) which was found only in uninoculated tobacco cells (<7 nmol g dry weight⁻¹ h⁻¹). The gas phase was replaced with a mixture of 20% O₂, 20% C₂H₂, 60% argon and after 2 h, the reduction of C₂H₂ to C₂H₄ was measured by gas chromatography. Acetylene reducing activity (nmol C₂H₄ per g dry weight per hour) was corrected for endogenous C₂H₄ production where applicable. The limit of resolution was 0.5 nmol per g dry weight per hour in 2 h assays.

†SH medium⁸ without phytohormones and with major salts replaced by (in mM) KH₂PO₄ (2.2), CaCl₂ · 2H₂O (0.7), KCl (0.9) and MgSO₄ · 7H₂O (0.3) and with decreased inositol (0.6) and added glutamine (10) and glycine (0.03), 10 ml media in 18 ml bottles.

‡SH medium without phytohormones and with KNO₃ and NH₄H₂PO₄ replaced by (in mM) NH₄NO₃ (1.5) and K₂HPO₄ (2.9) plus added glucose (50), 12 ml media in 28 ml bottles.

of plant culture media slants at 29°C in the dark. These and control cell cultures were assayed for acetylene-dependent ethylene production (Table 1) after 10 d.

Nitrogenase activity, indicated by C_2H_2 reduction, was appreciable in the 32H1-tobacco cell associations, and low but positive in the 32H1-cowpea cultures (Table 1). There was no C_2H_2 reduction by control plant cells or by 32H1 cultured alone on RN or GLN media, or by 32H1 on yeast-mannitol agar. The mean activity of all inoculated tobacco cultures was comparable to the activity reported for *Rhizobium*-soybean associations²⁻⁴, but there was less variability under our conditions. The rate of C_2H_2 reduction by six inoculated tobacco cultures on RN medium was linear for 6 h but declined by 40% after 20 h, activity in the two other replicates declined after 2 h.

There was copious growth of rhizobia in inoculated tobacco cultures and on the exposed and underlying surface of the agar slants. When the filter paper with inoculated cells was removed and discarded, the residual rhizobia on the slant maintained nitrogenase activity for 8 h at 2.2 nmol ethylene per culture per hour. This was 16% of the average value for undisturbed cultures.

The indication that the rhizobia possessed nitrogenase activity, and that activity depended on the presence of plant cells, was confirmed by growing a lawn of 32H1 on a GLN agar plate to within 5 mm of uninoculated tobacco callus. Bacterial growth was enhanced close to the tobacco callus, and after 7 d, agar segments (2.3 cm²) containing only bacteria, only callus, or neither, were excised and assayed. The segments with rhizobia reduced C_2H_2 at 0.8 nmol per segment per hour and activity was linear for 8 h, declining to 60% after 60 h. No activity was found in the other segments or in 32H1 cultured in the absence of plant cells.

Fourteen day old 32H1-tobacco cell associations on GLN agar slants were exposed to an atmosphere of 20% O₂ and 80% N₂, containing 80 atoms per cent ¹⁵N, for 28 or 42 h. The inoculated cells on filter paper and the underlying bacteria from the agar were removed separately, and ¹⁵N enrichment of the samples determined by mass spectrometry. The values for the cells were 0.034 and 0.039 (28 h) and 0.048 (42 h), while the corresponding values for the bacteria were 0.461, 0.612 and 0.483 atoms per cent excess compared with values of zero for identical material incubated in air. These highly significant ¹⁵N enrichments conclusively verify that the C_2H_2 -reducing activity observed in these experiments was due to nitrogenase.

The interest of these results depends on the evidence that our 32H1 culture was an uncontaminated strain of *Rhizobium* and that the plant cell suspensions were uncontaminated. Our culture of 32H1, initially purified by two successive single colony isolations, was slow-growing on yeast-mannitol agar and was both culturally and microscopically (Gram negative, motile rods) uniform. There was no growth, aerobically or anaerobically under N₂, on N-free or on yeast-peptone media, whereas *Klebsiella pneumoniae*, M5a1, grew rapidly under these conditions. Cowpeas growing in sterilised vermiculite-perlite were inoculated with a suspension of 10⁸ bacteria ml⁻¹, or a 10⁷ dilution of this, and produced nodules within 7 and 14 days, respectively, mature nodules reduced C_2H_2 . Bacteria reisolated from nodules were uniform, culturally identical to the parental 32H1, and formed C_2H_2 -reducing associations with tobacco cells. We verified the homogeneity of our 32H1 culture by producing both C_2H_2 -reducing associations with tobacco cells and nodules on cowpea with each of 15 single colonies isolated from the original culture, these isolates, cultured alone on GLN medium, did not reduce C_2H_2 . Only slow-growing bacteria similar to our parental 32H1 were recovered from each of the 15 inoculated tobacco cell suspensions. Uninoculated tobacco cell suspensions, cultured on yeast-mannitol, yeast-peptone, or under N₂ on N-free media, showed no evidence of bacterial contamination. We are confident that our 32H1 culture was a pure strain of

Rhizobium and that the tobacco suspensions were uncontaminated.

Our results indicate that the genetic information for nitrogenase is encoded in *Rhizobium*, as suggested by other evidence^{7,10,11}. Expression of the nitrogenase gene appears to depend on a diffusible factor secreted by plant cells.

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An attempt to separate fractions rich in human Y sperm

THE desire to predetermine sex before conception and thus alter the natural sex ratio has led many investigators to seek methods of separating the populations of X- and Y-bearing sperm and over the past 50 years, a number of claims of success have been made. Ericsson *et al.*¹ described a technique for the isolation of fractions rich in human Y sperm. On repeating the methods outlined in their paper, we were unable to find any evidence of enrichment of human Y sperm.

Initially, human semen was obtained from a hospital clinic, but there were two disadvantages, first, many of the samples showed poor sperm density and motility, and second, the time between ejaculation and reception of the semen at the laboratory was very variable. Later, an arrangement was made for seven anonymous volunteers to provide normal semen which could be used within 3-4 h of ejaculation. On arrival, all semen samples were examined on a warmed stage under a light microscope and assessed for percentage motility and degree of progressive motility. With a reasonable sample, 70-75% sperm showed progressive motility initially.

The separation technique was essentially that described previously¹ except that the fractions were obtained from the

Table 1 One-layer technique

Experiment no.	Normal semen smear (control)			Sperm from 6% BSA fraction		
	Total no of sperm counted	No of Y sperm counted	Y sperm (%)	Total no of sperm counted	No of Y sperm counted	Y sperm (%)
1	322	106	32.9	306	99	32.4
2	306	123	40.2	413	141	34.1
3	309	121	39.2	541	203	37.5
4	366	113	30.9	423	113	26.7
5	320	94	29.4	422	110	26.1

Table 2 Two-layer technique

Experiment no	Normal semen smear (control)			Sperm from 6% BSA fraction			Sperm from 15% BSA fraction		
	Total no of sperm counted	No of Y sperm counted	Y sperm (%)	Total no of sperm counted	No of Y sperm counted	Y sperm (%)	Total no of sperm counted	No of Y sperm counted	Y sperm (%)
6	126	46	36.5	121	52	43.0	43	18	41.8
7	413	143	34.6	200	72	36.5	155	56	36.1
8	120	51	42.5	299	111	37.1	155	47	30.3
9	210	79	37.6	209	84	40.2	200	85	42.5
10	209	83	39.7	218	64	29.4	104	35	33.7
11	221	99	44.8	212	84	39.6	214	72	33.6
12	313	153	48.9	129	50	38.8	183	65	35.5
13	504	200	39.7	629	150	23.8	593	150	25.3
14	618	254	41.1	333	101	30.3	250	56	22.4

bottom of the column. The semen was prepared by diluting 1:1 (v/v) with Tyrode's solution, centrifuging at 2,500g for 15 min and then resuspending the sperm in Tyrode's solution to give a final sperm density of approximately $100 \times 10^6 \text{ ml}^{-1}$. The semen was kept at room temperature throughout.

Columns of bovine serum albumin (in Tyrode's solution) were prepared in Pasteur pipettes (internal diameter 5.7 mm), closed by a clip on soft polythene tubing at the point of tapering. By controlling the clip, the flow of liquid from the column was easily regulated. Three separation procedures were used.

(1) One-layer technique. Each column contained 0.9 ml bovine serum albumin (BSA) in Tyrode's solution, of set concentration—a 6% solution was used in this series of experiments. The resuspended sperm (0.5 ml) (approximately 50×10^6 sperm) in Tyrode's solution were layered carefully on top of the albumin solution and left for 1 h. The top sperm layer was then removed with a pipette and the lower albumin fraction (isolation fraction) was removed from the base of the column. The sperm were recovered from the albumin by centrifugation at 2,500g and washed once in Tyrode's solution. The sperm were then resuspended in a little Tyrode's solution and smears made for staining.

(2) Two-layer technique. This was the modification described by Ericsson whereby discontinuous gradients of albumin solutions were prepared within the same Pasteur pipette column. Fifteen per cent BSA solution (0.4 ml) was overlaid with 0.8 ml 6% BSA solution. Sperm, prepared and resuspended in Tyrode's as before, was applied carefully to the top of the column. The column was left for 1 h, then the top sperm layer was pipetted off and the column was left for a further 30 min before the separate albumin layers were drawn off slowly from the base of the column. The separate BSA fractions were centrifuged at 2,500g and the sperm washed in Tyrode's solution (usually only one washing) before smears were made for staining.

All smears were air-dried and fixed in methanol for 30 min. The smears were stained with 0.5% aqueous solution of quinacrine dihydrochloride (Atebrin, Gurr) for 8 min followed by a brief tapwater rinse and a brief deionised water rinse, before mounting in water with a sealed coverslip. The smears were examined under oil with a dark field condenser at $\times 1,000$ magnification, using a Leitz microscope with a mercury vapour lamp and BG12 and 38 exciter filters. The total number of sperm counted was around 200 per sample which is the number used in our routine procedure for assessment of sperm quality and morphology. The proportion of these with a fluorescent spot or F body within the sperm head was noted. In a study of this nature, the staining procedure is of paramount importance. We investigated the effect of the length of time on staining and found that 8 min gave us the most consistent results. The assessment of the Y sperm was done very critically in that, if there was any doubt at all as to the presence of an F body, it was assumed to be absent. Such rigorous assessment of the Y sperm would undoubtedly lower the apparent percentage present.

As an experimental control, smears were made of untreated semen before preparing the sample for column separation. These smears were fixed, stained and examined at the same time as the experimental smears. We also investigated the effect of BSA on the staining process and in our hands we found that the presence of BSA did not affect the staining and difficulty was only encountered when we attempted to use 20% BSA.

Tables 1–3 show the results of 19 experiments in which a total of 14,374 sperm were counted. The presence of a fluorescent spot within the sperm head was taken to denote a Y-bearing sperm.

As can be seen from the tables of results, no positive increase in the proportion of Y sperm was achieved by passing the sperm through columns of BSA solutions. It might seem from the results that there is an increase in the number of X sperm. This is most likely because of the rigorous criteria which we applied to

Table 3 Three-layer technique

Experiment no	Normal semen smear (control)			Sperm from 6% BSA fraction			Sperm from 10% BSA fraction			Sperm from 15% BSA fraction			Sperm from 20% BSA fraction		
	Total no of sperm counted	No of Y sperm counted	Y sperm (%)	Total no of sperm counted	No of Y sperm counted	Y sperm (%)	Total no of sperm counted	No of Y sperm counted	Y sperm (%)	Total no of sperm counted	No of Y sperm counted	Y sperm (%)	Total no of sperm counted	No of Y sperm counted	Y sperm (%)
15	238	93	39.0	205	72	35.1	—	—	—	209	106	50.7	154	67	43.5
16	135	56	41.5	60	30	50.0	—	—	—	150	30	20.0	105	28	26.7
17	209	81	38.8	—	—	—	64	29	45.3	108	40	37.0	20	6	30.0
18	210	67	31.9	—	—	—	115	17	14.8	288	89	30.9	190	66	34.7
19	316	110	34.8	—	—	—	328	85	25.9	288	71	24.7	273	78	28.6

(3) Three-layer technique. The column was prepared by layering decreasing gradients of 20%, 15% and 10% or 6% BSA solutions over one another in 0.3 ml volumes. The top layer was 0.5 ml resuspended sperm in Tyrode's solution. The column was left for 1 h before the top layer was pipetted off and then for a further 60 min before the albumin fractions were drawn off. The sperm were removed from the separate BSA fractions by centrifugation at 2,500g and usually washed once in Tyrode's solution before smears were made for staining.

the identification of the Y sperm and does not indicate an actual increase in the number of X sperm.

Separation is dependent on the progressive motility of the sperm. We encountered difficulty in resuspending the sperm evenly after centrifugation. The process of washing the sperm and removing the seminal plasma resulted in a reduction in the percentage of progressively motile sperm in the first layer of the column. Clumps of sperm were formed which were so large that they could be seen to fall through the albumin layers under

gravity. The multiple layer techniques seemed to be preferable to the one-layer procedure because sperm were not subjected to centrifugation and resuspension before being placed on further albumin columns to complete the separation.

We have been unable to confirm the results obtained by Ericsson *et al*¹. The crucial parts of the procedure are the staining process and the subsequent identification of Y sperm. The standardisation of our staining technique and the criteria which we applied to the identification of the Y sperm are not responsible in our opinion for the negative results which we have obtained.

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Failure to confirm separation of X- and Y-bearing human sperm using BSA gradients

THE ability to predetermine the sex of offspring before conception would have significant clinical and sociological implications in man¹ and would have great economic benefit if applied to the breeding of livestock. The claim of Ericsson *et al*² that human spermatozoa bearing Y chromosomes can be separated from sperm containing X chromosomes by passage through a medium containing bovine serum albumin (BSA) or ovalbumin, is therefore of some importance. We have attempted to repeat the experiments to confirm these findings and report here our failure to do so.

The experiments of Ericsson *et al*² yield two important conclusions. First that it is possible to separate the more motile fraction of human spermatozoa by layering a suspension of sperm in Tyrode's solution over various concentrations of BSA in single or multiple steps, when the more motile sperm enter the layers of higher concentration while the less motile sperm are retained within the Tyrode's solution or the less dense layers. Second, that sperm selected for increased motility in this way turn out to be predominantly Y-bearing sperm for example in two experiments where sperm motility was

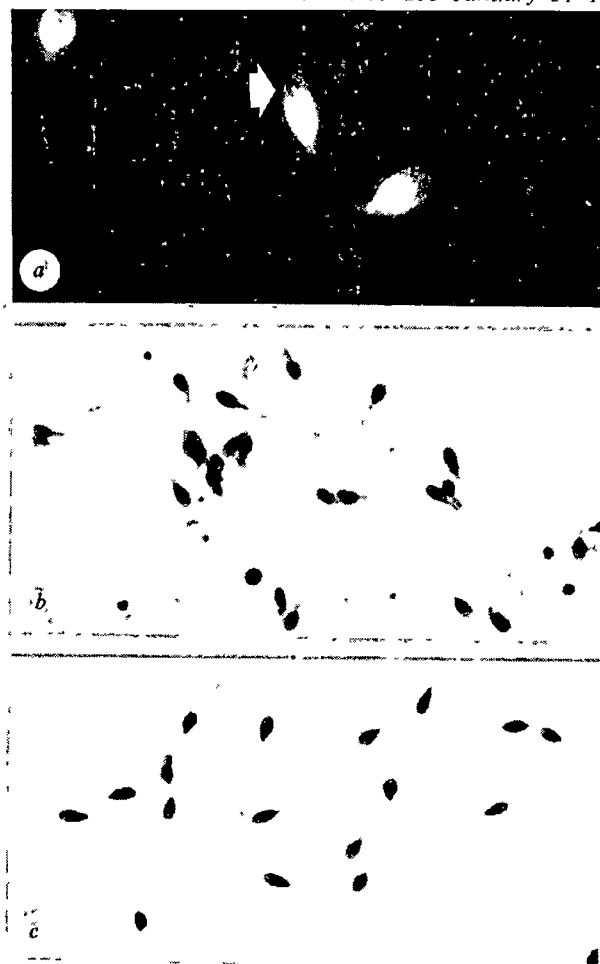


Fig 1 *a*, Quinacrine stained spermatozoa showing the presence (arrowed) or absence of the fluorescent Y body. *b*, Spermatozoa from sample 5 before layering over BSA, showing the heterogeneous morphology of the sperm heads. *c*, Spermatozoa from sample 5 after layering over BSA (final isolate) showing a homogeneous morphology of sperm heads.

increased from around 65% to 98%, the percentage of Y-bearing sperm was reported to have increased from around 50% to 80%. In these experiments, the sex chromosome status of spermatozoa was ascertained by scoring for the presence or absence of a brightly fluorescing Y body (equated with the Y chromosome, see Fig 1*a*) in samples stained with quinacrine dihydrochloride³⁻⁵. Our work confirms the first conclusion but not the second.

A series of initial experiments was performed to test the effects of various concentrations of BSA and of different fixation procedures which might influence the fluorescent staining and Y-body detection in spermatozoa. It was found

Table 1 Sperm motility (%) and morphology*

Individual (Sample no.)	Control untreated (a)	Washed in Tyrode's and centrifuged (b)	Top layer	10% BSA	15% BSA	25% BSA
1	50 (25)	30	1			80 (50)
2	40 (—)	20	10			70 (—)
3	70 (67)	40	40	1	10	50 (77)
4	80 (51)	20	2	5	12	80 (78)
5	60 (51)	60	2	7	70	90 (68)
6	80 (64)	80	2	30	80	100 (84)
7	30 (22)	30	1	2	25	75 (88)
8	55 (20)	50	4	7	20	89 (94)

* Figures in parentheses are estimates (%) of sperm with normal morphology based on 100 spermatozoa per sample. The remaining entries are data on sperm motility based on a minimum of 100 sperm per sample.

The difference in motility (%) between (a) and (b) in samples 1-4 is the result of centrifugation in Tyrode's at 1,750g. There is no difference in motility (%) between sperm in untreated semen and samples washed and centrifuged at 800g, compare (a) and (b) for samples 5-8.

Table 2 Percentage of sperm with Y bodies

Individual (Sample no)	Washed sperm Control		Top layer		10 %BSA		15% BSA		25 % BSA	
	No of sperm counted	Y sperm (%)	No of sperm counted	Y sperm (%)	No of sperm counted	Y sperm (%)	No of sperm counted	Y sperm (%)	No of sperm counted	Y sperm (%)
1*	200	49	200	43					200	41
2*	200	47	200	51					200	44
3	200	45	200	40	200	57	200	49	200	48
4	200	45	200	48	200	48	200	45	200	44
5	200	41	200	48	200	45	200	31	200	46
6	400	49	400	51	400	51	400	51	400	50
7	400	45	400	45	400	45	400	44	400	44
8	400	46	400	49	400	48	400	46	400	46
Total	2,200	46	2,200	47	1,800	49	1,800	45	2,200	46

* The samples from subjects 1 and 2 were subjected to a single-step and the remainder to a three-step isolation procedure

that the efficiency of detecting a Y body was not influenced by BSA concentrations over a range of 6–25%, although higher background fluorescent levels were observed at the higher concentrations even though the samples had been washed four times in Tyrode's solution. Better fixation and staining was obtained from suspensions of sperm fixed in methanol acetic acid (3:1) than in methanol alone.

In the experiments reported here, semen samples were obtained from each of five healthy volunteers and three males attending a subfertility clinic. Each sample was subjected to either the simplest single-step, or the most efficient three-step continuous gradient, described by Ericsson *et al.*² In all cases the sperm count, volume and motility were estimated on the fresh specimen and a smear was prepared for morphological studies. The remainder of the sample was washed in Tyrode's solution (1:1) and centrifuged at 4,000 r.p.m. (1,750g) for 15 min and resuspended in Tyrode's solution at a concentration of 1×10^8 sperm ml⁻¹.

In the first four specimens studied it was noted that sperm motility was reduced after washing and centrifugation at 1,750g (see Table 1) and further experiments were carried out to examine possible effects of centrifugation on sperm motility in Tyrode's solution. It was found that 800g (2,000 r.p.m.) was the maximum centrifugal force with no adverse effect on sperm motility in Tyrode's solution and this was used in the later experiments. This effect on motility was only observed after centrifugation of sperm in Tyrode's solution and was not apparent when sperm were centrifuged in BSA/Tyrode's solutions.

Single-step gradients were set up in Pasteur pipettes by layering 0.5 ml of a suspension containing 1×10^8 sperm ml⁻¹ on to the top of 0.9 ml of 25% BSA (ref. 2). Continuous gradients consisting of 0.3 ml of 10%, 15% and 25% BSA as described by Ericsson *et al.*² were layered on top of each other in Pasteur pipettes and then 0.5 ml of the sperm suspension was carefully layered on top. The columns were left at room temperature for 1 h when the top layer (Tyrode's plus sperm) was removed by pipette. In the continuous gradients the 10% BSA layer was pipetted off after a further 30 min and the final layers after another 30 min. In the single gradients the top layer and the final layer were removed after 1 h. After removal, each layer was washed four times in Tyrode's solution and fixed in methanol acetic acid (3:1). The fixed suspensions were dropped on to slides and air dried before staining in an aqueous solution of quinacrine dihydrochloride for three minutes. All slides were coded, randomised and scored blind for the presence of a Y body in 100 or 200 sperm from each of two slides made from each layer of each gradient. The observations were made using a Leitz Ortholux microscope fitted with the appropriate fluorescence attachments⁶.

Our results show that the percentage of Y-bearing sperm in the final isolates does not deviate significantly from that of the controls (Table 2) and thus contradict those of Ericsson *et al.*² They do, however, demonstrate that isolation seems to be dependent on sperm motility and that as Ericsson *et al.*²

describe, the non-motile sperm are initially retained in the upper less dense layers (Table 1).

It is well known that spermatozoa in a semen sample from normal fertile human males, in contrast to most other mammals, show considerable morphological heterogeneity⁷ and include a significant proportion of immature cells. The percentage of so-called 'normal' forms (defined largely on shape and structure of head and tail) is, in our laboratory, very often below 50% and rarely above 70%. As Ericsson *et al.*² have noted, selection for motility also results in increasing the proportion of morphologically normal sperm. Our own data (Table 1) clearly support and confirm this conclusion (compare with Fig. 1b and c). With this technique, however, the yield of normal motile sperm is low (between 1% and 5% of the original sperm input) and, although dependent on the quality of the initial sample, could doubtless be increased with improved methods.

There is no doubt, therefore, that fractionation of sperm samples using BSA gradients provides a method for selecting morphologically normal sperm of high motility so the technique might prove useful in the treatment of male subfertility. A series of ejaculates could be treated by the BSA method and the isolates stored in liquid nitrogen until a sufficient sperm concentration is achieved.

Finally we emphasise that our results do not support the contention that there is a differential motility between X- and Y-bearing spermatozoa that can be exploited to separate these cell types using the methods described by Ericsson *et al.*²

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Sex and age differences in human platelet aggregation

MALES stand at risk in many pathological states¹, especially cardiovascular disease, in which there is a statistically significant higher mortality in men than women². We have described³ a sex difference in platelet responsiveness in rats and guinea pigs where platelets from males were significantly more sensitive to aggregating stimuli than those from females. Increased platelet responsiveness has been detected in humans,

in thromboembolic disease⁴ and after acute myocardial infarction⁵. Indeed, the principal *post mortem* finding in subjects who died suddenly is occlusion in the pulmonary circulation due to platelet aggregation⁶. We have suggested³ that platelet sensitivity is mediated by the androgenic steroids and that androgens contribute to the incidence of thrombotic disease.

In contrast to the situation with synthetic contraceptive steroids⁷, few variations have been observed in the response of human platelets to endogenous sex hormones, although adenosine diphosphate (ADP)-induced aggregation in women was increased during ovulation and in pregnancy⁹. Zahavi *et al.*⁸ reported that platelets from normal women on the second day of menstruation were more responsive to aggregation than those from normal men. Platelet adhesiveness in the presence of an encephalitogenic factor was also greater in women than men¹⁰, although this was not observed in another study where platelet adhesiveness was estimated directly¹¹.

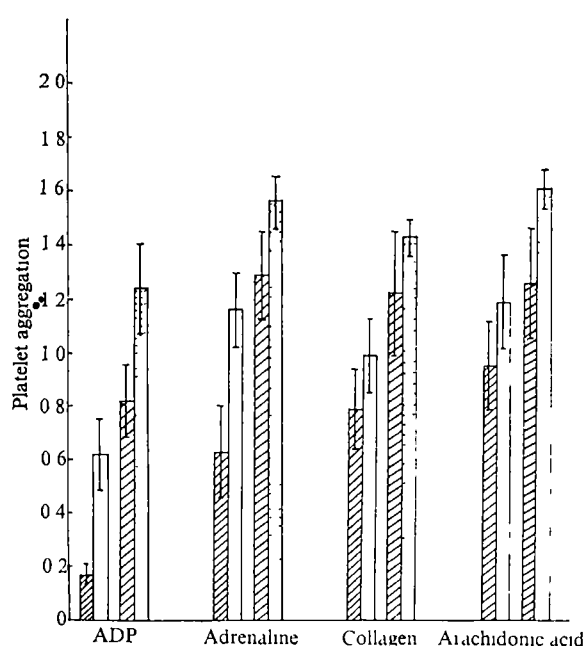


Fig. 1 Effect of sex and age on human platelet aggregation. PRP was prepared from the blood of human male, mean age 23.2 (hatched symbol), mean age 42.4 (hatched/dotted symbol) and female donors, mean age 25.2 (open symbol), mean age 45.9 (dotted symbol), at the same time of day²¹. The platelet count was adjusted to $\sim 200,000 \text{ mm}^{-3}$ with autologous platelet-poor plasma (PPP). The light transmission through PRP and PPP was taken as 0% and 100% aggregation respectively. The extent of maximum aggregation (T_{max} ¹⁷, in light transmission units) was recorded following addition of an aggregating agent to 1 ml of PRP (37°C , 900 R.P.M.) in a siliconised tube. The response to ADP (Equine muscle, Sigma, $1 \mu\text{g ml}^{-1}$), adrenaline (Parke Davis, $10 \mu\text{M}$), collagen ($50 \mu\text{l}$ of suspension)²² and arachidonic acid²³ (1 mM) in 35 estimations ($\pm \text{s.e.m.}$) is indicated.

The mortality rate from cardiovascular disease increases exponentially with age in both men and women, although it is always higher in men and reaches a maximum in the fifth decade of life¹². We have now found that platelet sensitivity to aggregant stimuli similarly increases with age, especially in men, and that donors classified as high mortality risk (on an age basis) have enhanced platelet responsiveness when compared with younger donors of lower risk.

Blood samples were drawn from the cubital vein of resting human volunteers into 3.8% (w/v) trisodium citrate (1 part anticoagulant to 9 parts blood). Donors were screened for recent history of medication¹³, cigarette smoking¹⁴ and no samples were taken within 3 h of a meal¹⁵. Platelet-rich plasma (PRP) was prepared as described previously¹⁶ and maximum aggregation (Fig. 1) was assessed routinely 1 h after sampling, in an aggregometer (Payton Associates, New York). This

parameter is linearly proportional to platelet count and aggregating agent concentration¹⁷ and was observed here to give good reproducibility between repeated tests on the same donor. Primary aggregation, monophasic with pronounced disaggregation in response to ADP ($0.5 \mu\text{g ml}^{-1}$) and secondary aggregation, monophasic exponential or biphasic with minimal disaggregation in response to ADP ($5 \mu\text{g ml}^{-1}$), was demonstrated in each sample before use.

In one study, 19 healthy men, 21–25 yr old (mean 23.2) were compared with 19 healthy women, 21–26 yr old (mean 25.2). In a second study, 17 normal men, 38–47 yr old (mean 42.4) were compared with 16 premenopausal women, 36–50 yr old (mean 45.9). Samples from women were taken at random with respect to the menstrual cycle⁸, but not during menstruation. In a third study, postmenopausal women, 56–58 yr old (mean 57.0) were compared with normal men, 55–62 yr old (mean 58.5). These postmenopausal women were not on oestrogen supportive therapy. Mean values for the platelet response to various aggregating agents in these groups were compared, and the appropriate Student's *t* statistics calculated.

The primary aggregation (reversible) induced by ADP in platelets of young healthy women (mean age 25.2) was significantly ($P < 0.001$) increased over the values obtained in males (mean age 23.2) (Fig. 1). This female platelet sensitivity apparently depended on the degree of aggregating stimulus, and was 3.7 times greater than male sensitivity in response to ADP ($1 \mu\text{g ml}^{-1}$) and 2.0 times greater in response to ADP ($2 \mu\text{g ml}^{-1}$). These data are in accord with the report⁸ that female platelets were more responsive than male platelets to ADP ($0.6 \mu\text{g ml}^{-1}$ and $6.0 \mu\text{g ml}^{-1}$). They are, however, in contrast to our observations in the rat and guinea pig, where male platelet sensitivity was significantly greater than female sensitivity at concentrations of ADP from 0.1 to $10 \mu\text{g ml}^{-1}$. Primary and secondary (irreversible) aggregation induced by adrenaline was also significantly greater (1.8 times, $P < 0.01$) in human females than in males (Fig. 1). No significant difference was observed in secondary aggregation of these platelets induced by either collagen or arachidonic acid.

In the high risk age group (mean 45.7), platelet aggregation in response to primary and secondary aggregating stimuli was significantly ($P < 0.005$) enhanced in both males and females, when compared with the low risk group (mean 24.2). Female platelets were again more responsive (1.25 times) to ADP ($1 \mu\text{g ml}^{-1}$) than male platelets ($P < 0.05$) (Fig. 1). This female/male ratio of sensitivity was, however, significantly less than that in the low risk age group. Indeed, there was no significant sex difference in response to ADP ($2 \mu\text{g ml}^{-1}$) in the older group.

The change in sensitivity ratio was reflective of a significantly ($P < 0.01$) greater increase in male platelet responsiveness in the high risk group. For example, platelet aggregability increased 4.8 times in response to ADP ($1 \mu\text{g ml}^{-1}$) in males and only 2.0 times in females. Results were similar with adrenaline-induced aggregation. In the high risk group there was no significant difference ($P > 0.05$) between males and females in their response to adrenaline (Fig. 1). This is in contrast to the female/male ratio of 1.8 determined in the low risk group and represents a twofold increase in male platelet sensitivity.

The marked sex difference in platelet sensitivity observed in both high and low risk groups could be the consequence of an interaction of platelets with sex hormones. We have demonstrated that natural sex hormones may attenuate platelet behaviour *in vitro* and *in vivo*³. To determine the effects of oestrogen withdrawal¹⁸, platelets from premenopausal women (mean age 45.9) were compared with those from postmenopausal women (mean age 57.0). There was no significant difference in platelet responsiveness to either ADP or adrenaline between these groups, suggesting that oestrogens are not responsible for the enhanced platelet sensitivity in females.

In addition, incubation of male or female human PRP with oestradiol ($1 \mu\text{g ml}^{-1}$) or with progesterone ($1 \mu\text{g ml}^{-1}$), under conditions which detect possible platelet-sensitising effects of

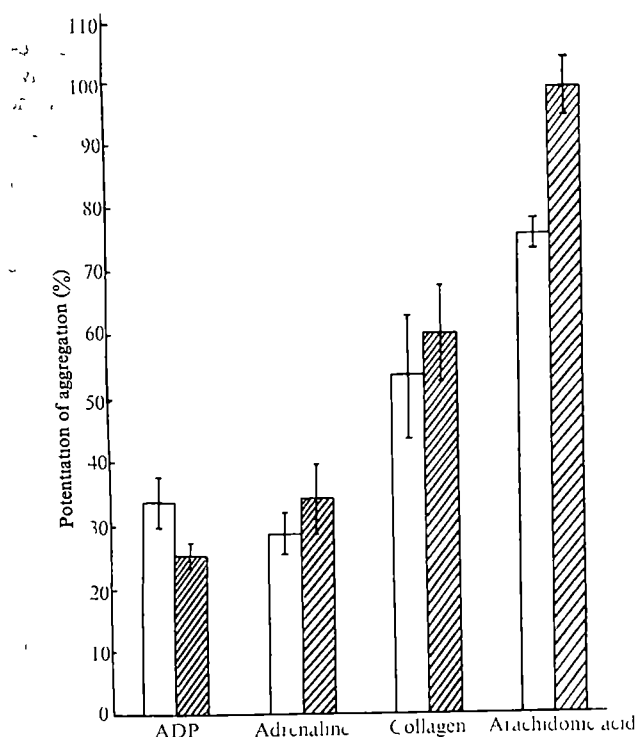


Fig 2 Effect of testosterone *in vitro* on human platelet aggregation. Human male (open symbol) and female (hatched symbol) PRP (platelet count $\sim 200,000 \text{ mm}^{-3}$) was incubated in the presence or absence of testosterone ($1 \mu\text{g ml}^{-1}$) for 30 min at 22°C . Potentiation (%) of aggregation to ADP ($1 \mu\text{g ml}^{-1}$), adrenaline ($10 \mu\text{M}$), collagen ($30 \mu\text{l}$ of suspension) or arachidonic acid (1 mM) over that in the control (vehicle-treated) plasma is illustrated. The mean of 25 estimations ($\pm \text{s.e.m.}$) is indicated.

the sex hormones (M J, E R, and P W R unpublished) resulted in only 10.4 ± 2.4 and $10.9 \pm 0.3\%$ increases in responsiveness to ADP. This was significantly ($P < 0.01$) less than after incubation with androgenic steroids. Testosterone ($1 \mu\text{g ml}^{-1}$) enhanced the aggregating capability in response to ADP by 33.6 ± 3.9 and $24.9 \pm 2.1\%$ in male and female platelets respectively (Fig 2). It is interesting that testosterone was also more effective than either oestradiol or progesterone in inducing sensitivity to secondary (irreversible) aggregating agents such as adrenaline, collagen and arachidonic acid. Aggregation of both male and female platelets induced by arachidonic acid was very responsive to androgen *in vitro*, exhibiting 75.0 ± 1.9 and $97.0 \pm 4.5\%$ increases in sensitivity respectively (Fig 2).

Thus we have demonstrated that (a) there is a significant sex and age difference in human platelet sensitivity to aggregating stimuli, (b) female platelets are more responsive than the corresponding male platelets, (c) platelet responsiveness in both sexes increases with age, male sensitivity increasing at a greater rate than female sensitivity, and (d) cardiovascular disease high risk age groups exhibit enhanced platelet responsiveness when compared with low risk groups.

The sex difference in platelet sensitivity may be intrinsic to the platelet. Our evidence indicates, however, that natural oestrogenic and progestogenic steroids have minimal effects on platelets both *in vivo* and *in vitro*, although androgenic steroids were significantly active *in vitro*. Platelet sensitivity may equally be determined by interaction with various clotting factors, fibrinogen, plasminogen and fibrinolytic inhibitors, the levels of which are sex hormone-dependent¹⁹.

Disaggregation⁵ and adhesiveness¹¹ of platelets is apparently not related to the age of the donor. Male platelet responsiveness, however, increased significantly with age and concurrent with other androgen-related risk factors such as pressor sensitivity²⁰, blood viscosity¹ and serum cholesterol, phospholipid and tri-

glyceride levels¹² may contribute significantly to the incidence of arterial disease.

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Endometrial cell calcium and oestrogen action

ADMINISTRATION of oestrogen to an ovariectomised or immature rat promotes a rapid increase in the content of water and univalent electrolytes in uterus and vagina^{1,2}. Although alterations in the uptake and retention of calcium and other divalent cations by several hormone-responsive tissues have been reported following long term administration of oestrogen *in vivo*³⁻⁵ and during the oestrous cycle⁶, no information is available on the cellular exchange, if any, of calcium shortly after administration of oestrogen in sensitive targets. In view of evidence suggesting a critical role for calcium in the initiation and/or regulation of cell growth and metabolism⁷, consideration of the potential involvement of this cation in the uterine response to oestrogen is important to analysis of steroid hormone action at the cellular level. Using endometrial cell suspensions isolated from the uteri of ovariectomised rats⁸, we have now shown that physiological levels of oestradiol-17 β influence rates of cellular calcium exchange as early as 2.5 min after *in vitro* addition of hormone.

Endometrial cells were isolated and maintained as before⁸ and suspended in Ringer solution composed of 136.9 mM NaCl, 2.7 mM KCl, 0.7 mM CaCl₂, 0.6 mM MgCl₂, and buffered at pH 7.4 with 8.1 mM Na₂HPO₄/1.5 mM KH₂PO₄, in the presence of 1 mM Na pyruvate. Calcium uptake by the isolated endometrial cells was determined from accumulation of ⁴⁵Ca²⁺ (New England Nuclear Corp.) A 40-ml cell suspension ($\sim 10^6$ cells per ml) in a 50-ml plastic Erlenmeyer flask was placed over a submersible magnetic stirrer in a water bath at 37°C and gassed with O₂. After a brief equilibration period, 50 μCi of ⁴⁵CaCl₂ was added (zero time) and incubation continued. Samples of medium were taken at selected intervals after addition of the isotope. For each time interval, two samples (1.5 ml each)

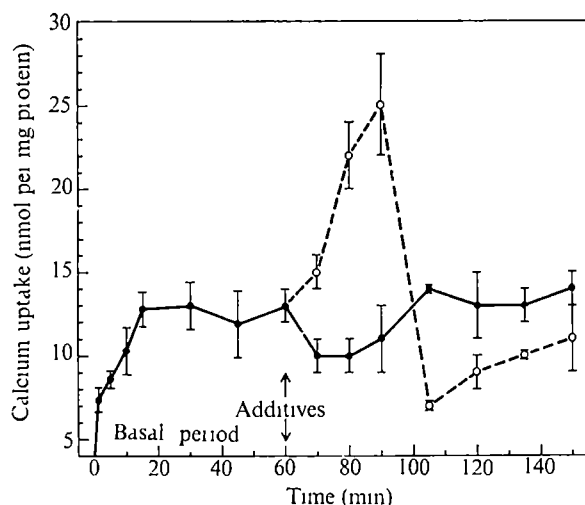


Fig 1 Calcium uptake of isolated endometrial cells is expressed as (d.p.m. per mg cell protein)/(d.p.m. per nmol medium calcium). After a 60 min basal period, oestradiol-17 β (○), to a final concentration of 1×10^{-8} M, or its vehicle, ethanol (●), to a final concentration of 0.02%, were added *in vitro* to experimental and control suspensions, respectively. The incubations were then continued for 90 min. Each point (mean \pm s.e.m.) represents data from three to six independent experiments.

were removed and transferred into two plastic centrifuge tubes containing 11 ml ice-cold, isotonic choline chloride, buffered at pH 7.4 with Tris. The tubes were immediately centrifuged (at 4°C in a Sorvall SC2B centrifuge) at 600g for 1 min. The supernatant was decanted, and the tubes were inverted to drain overnight. Double-distilled deionised water (4 ml) was added to the cell sediment which was homogenised with a high-intensity ultrasonic probe. Aliquots of sonicate were taken for determination of cell protein⁹ and ^{45}Ca by liquid scintillation counting. Oestradiol-17 β , where indicated, was added to the medium to a final concentration of 1×10^{-8} M. 60 min after zero time, the control flask received vehicle alone.

Efflux of ^{45}Ca was determined with cells preloaded with isotope by the addition of $^{45}\text{CaCl}_2$ (final concentration of $50 \mu\text{Ci ml}^{-1}$), followed by 60 min of incubation. The suspension was washed by centrifugation twice with buffer for 1-min periods at 600g. The final sediment was resuspended in 6 ml of ^{45}Ca -free Ringer solution and one-half distributed into each of two stop-flow chambers. The chambers, made from polycarbonate, were mounted on a filter funnel equipped with a stopcock which could be opened for rapid filtration of the suspension medium through a 0.45- μm Millipore filter. The top of the chamber was sealed with a rubber serum cap through which O_2 and wash solutions could be introduced by means of syringe needles. This assembly was immersed immediately above a submersible stirring magnet in a water bath at 37°C. A 1 mm bar magnet stirred the suspension continuously.

Isotope desaturation began with the addition of cell suspensions to the chambers. The medium was replaced at selected times, when cells were washed with 2×3 ml 'cold' Ringer solution with the stopcock open. The stopcock was then closed and 3 ml of fresh medium was delivered to resume the desaturation. Washing was completed within 30 s. Aliquots of the spent media were taken for liquid scintillation counting of ^{45}Ca . After desaturation, cells were collected and sonicated as above. Aliquots of sonicate were taken for determination of total cell ^{45}Ca and protein as above.

• Figure 1 shows that from zero time to 60 min, accumulation of calcium in the cells in the absence of test agents was exponential, similar to that described for other mammalian cells¹⁰. Addition of oestradiol increased Ca^{2+} uptake to 153% of control levels in 10 min ($P < 0.05$) with a peak

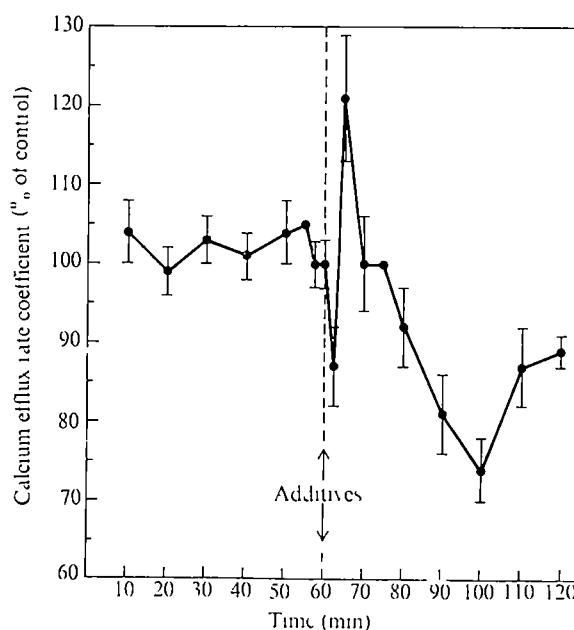
at 230% of controls ($P < 0.01$) within 30 min. Uptake declined to 50% of control values ($P < 0.01$) by 45 min and then gradually rose towards the baseline.

To test whether oestrogen increased net calcium accumulation in part by inhibiting efflux, we measured calcium efflux rate coefficients in the presence and absence of oestradiol-17 β , added *in vitro*. Cells were preloaded with isotope for 60 min and then distributed equally into an experimental and a control group. Figure 2 shows that during the first hour of desaturation, represented by the basal period, efflux occurred at essentially the same rate in both samples. After addition of oestradiol at 60 min, however, $^{45}\text{Ca}^{2+}$ efflux began to oscillate both above and below control levels. It declined to 87% of control after 2.5 min ($P < 0.05$), rose to 121% of control within 5 min ($P < 0.05$) and then declined to its lowest ebb, at 74% of control ($P < 0.001$), 40 min after addition of hormone. Thereafter, it remained depressed ($P < 0.05$) to the termination of desaturation measurements at 120 min.

The relationship of these early changes in calcium flux to later metabolic effects of oestradiol was considered in further experiments. We have shown previously that cortisol can block the stimulatory effect of oestradiol on glucose metabolism of isolated endometrial cells⁸. The action of 3×10^{-8} M cortisol hemisuccinate (Solu-Cortef, Upjohn) and its interaction with 1×10^{-8} M oestradiol in affecting $^{45}\text{Ca}^{2+}$ accumulation were evaluated from measurements of calcium uptake taken for 30 min after addition of steroids to isolated cells, ethanol to a final concentration of 0.02% was added to suspensions of control cells. Cortisol alone did not affect Ca^{2+} uptake, for in its presence uptake was 95% of controls. Addition of cortisol immediately before exposure to oestradiol, however, abolished the expected increase of calcium influx within 30 min (94% of control). These results parallel those obtained for intestinal calcium transport which indicate that glucocorticoids antagonise vitamin D-induced absorption of calcium¹¹.

Our experiments demonstrate that physiological concentrations of oestradiol-17 β act *in vitro* to produce oscillations

Fig 2 The calcium efflux rate coefficient of isolated endometrial cells was calculated according to Isaacson and Sandow¹⁵. With the rate coefficient of the control group being taken as 100%, the changes in the oestradiol-treated group at each point were expressed as percentage of control. Additives were introduced *in vitro* at the concentrations indicated in Fig 1 after a 60-min basal period. Each point (mean \pm s.e.m.) represents data from three to seven independent experiments, points without s.e.m., however, are taken from the results of two experiments.



in the net calcium content of isolated endometrial cells by influences on rates of influx and efflux. Similar variations in the cellular content of calcium have been considered important in the action of other hormones¹². Other evidence also indicates that increments in cellular calcium accumulation precede and/or trigger cell division and growth^{6,7}, which is the end-point of oestrogen action in endometrium. Strict control of intracellular calcium seems to be achieved by regulation of fluxes across the plasma membrane and between the cytoplasm and subcellular organelles, such as mitochondria¹¹. It has also been reported that diethylstilboestrol blocks energy-dependent Ca^{2+} uptake by mitochondria isolated from the myometrium¹³. Collectively, these oestrogenic effects on calcium exchange must lead to marked changes in cytoplasmic Ca^{2+} activity, binding and redistribution at intracellular sites. The characterisation of calcium exchange in resting and oestrogen-stimulated endometrial cells, therefore, has significant bearing on the processes involved in metabolic and mitogenic responses to the hormone¹⁴.

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Two biological activities regulating cell proliferation found in cultures of peritoneal exudate cells

WE have found two distinct biological activities in cultures of peritoneal exudate cells rich in macrophages. One consists of an inhibitory effect on cell proliferation, described previously, caused by a low molecular weight product¹, the other is a stimulatory effect brought about by a non-dialysable material. Both activities were found in the same culture fluid, and different kinds of cells exhibited different sensitivities to them. Our results offer a possible explanation for the discrepancies in the literature concerning the different effects of macrophages or their products on cells¹⁻⁷.

We cultured the peritoneal exudate from A/St or C57BL/6 mice injected 3 d previously, intraperitoneally, with 1.5 ml of a 10% (v/v) solution of proteose peptone (Difco). The cells were plated at a density of 10^7 or 2×10^6 cells per ml on 35 mm plastic dishes (Falcon) in RPMI-1640 culture medium supplemented with 5% foetal calf serum. After 24 h, the medium was collected, the cell monolayer

was washed, fresh medium was added, and the culture continued for 48 h. The activities of the culture fluids obtained during the first 24 h or from the 24-72-h period were tested. Most cells forming the exudate were typical macrophages as judged morphologically, during the first 24 h, 5-10% lymphocytes were present with the macrophages, but most were eliminated by the washing at 24 h. We tested the incorporation of ^3H -thymidine by EL-4 leukaemia cells, P815 mastocytoma cells, thymocytes and spleen cells cultured in the presence of various amounts of fluids from macrophage cultures. Results have been consistent for more than twenty different experiments. The main results shown in Figs 1-3 were obtained with a fluid from a culture of 10^7 cells per ml.

Incorporation of ^3H -thymidine into EL-4 leukaemia cells cultured for 8 h in the peritoneal cell supernatant was inhibited markedly (Fig 1). This inhibition correlated with that of cell mitotic activity and with the increase of cell density with time of culture¹. The culture fluid, if dialysed extensively against normal culture medium, lost all inhibitory activity. EL-4 cells cultured in dialysed culture supernatants incorporated the same amount of thymidine as cells cultured in normal medium. Another cell line, the mouse mastocytoma P815 used extensively in current *in vitro* studies of cell-mediated immunity, behaved as the EL-4 line, and its incorporation was inhibited markedly by the supernatants (data not shown).

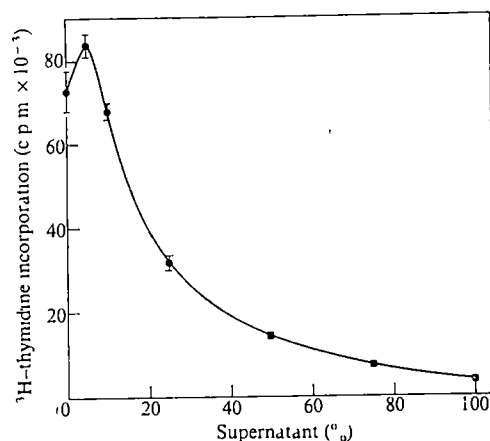


Fig 1 Incorporation of ^3H -thymidine by EL-4 leukaemia cells cultured in the presence of different amounts of culture fluids from peritoneal exudate cells. Cells were cultured at a density of 5×10^5 per ml in plastic tubes for 8 h, the last 4 h in the presence of ^3H -thymidine (New England Nuclear Corp., 2 Ci mmol⁻¹). Each result is the mean of three culture tubes. Brackets refer to standard error of the mean. In this experiment, the EL-4 cells (a tumour line from C57BL/6 mice) were cultured in the presence of fluids from A/St mice. Identical results have been obtained if the cells are cultured in fluids from C57BL/6 peritoneal cells.

We examined for the amounts of inhibitor extracted by freeze-thawing the peritoneal exudate cells or released into the medium after culture for 24 h. The amounts of material (v/v) required to reduce by 50% the incorporation of ^3H -thymidine by EL-4 cells were determined. The amounts were 63% from cells lysed immediately after plating, 83% from cells lysed 24 h after plating, and 22% from the fluids after culture for 24 h. Thus, the inhibitory activity found in culture fluids was generated during the culture and did not result from lysed or dead cells. The chemical nature of this material is not known. In our previous experiment, we indicated that the material was resistant to tryptic digestion, to phosphodiesterase treatment, and to boiling and freeze-thawing¹.

The spontaneous incorporation of ^3H -thymidine into spleen cells cultured with the untreated supernatant was inhibited slightly. When these cells were cultured with

dialysed supernatants, c p m were the same as controls in the range of doses used, 10–50% (v/v) (In other experiments, a 75% concentration of the dialysed peritoneal cell supernatant increased incorporation of ^3H -thymidine by about 90%) If spleen cells were cultured in the presence of phytohaemagglutinin (PHA) for 3 d, there was an expected marked increase in incorporation (^3H -thymidine was present during the last 12 h of culture) (Fig 2) The culture conditions used here—3-d culture, 1640 medium with 5% foetal calf serum, 10^6 lymphocytes in 12×75 -mm plastic tubes, 12-h pulse of ^3H -thymidine, and 100 μl of 1:100 dilution of Difco PHA-P—were selected to produce optimal response The response to PHA was reduced markedly in the presence of peritoneal cell culture supernatant in agreement with our findings¹ The dialysed culture supernatant, however, not only lost its inhibitory property but increased the response of the spleen cells to PHA This experiment, therefore, revealed two activities differentiated on the basis of size, influencing the multiplication of spleen lymphocytes

Of particular interest are the results with thymocytes The conditions for thymocyte culture were identical to those used for spleen cells, except for the cell density, which was 5×10^6 cells per ml First, the untreated supernatants increased slightly the spontaneous incorporation of thymidine into thymic cells, the dialysed fluid, however, had markedly more activity (Fig 3) Clearly, the fluids contained a principle that stimulated ^3H -thymidine incorporation and which was partially masked by a low molecular weight inhibitor Thymocytes responded poorly to PHA as expected For example, the spontaneous c p m of thymocytes after culture for 3 d was 678 (mean of triplicate culture, s e m ± 115), PHA for 3 d increased this only to 1,337

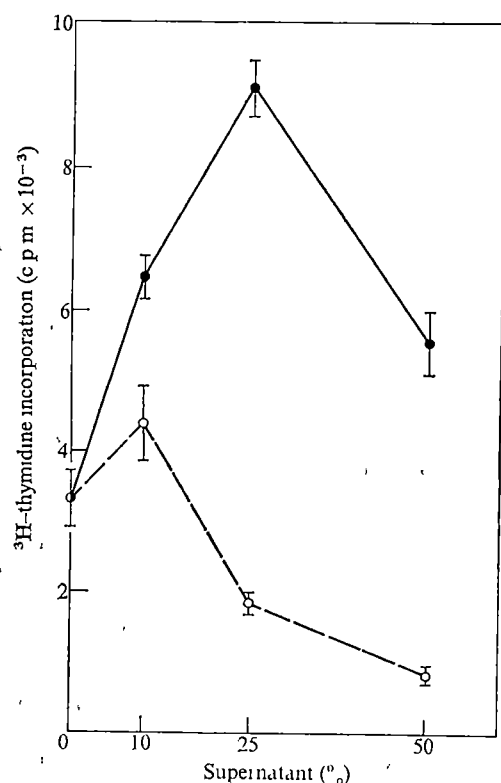


Fig. 2 Spleen cells from A/St mice were cultured in the presence of various amounts of the supernatants and PHA Time of culture was 72 h, the last 12 h in the presence of ^3H -thymidine Cultured fluids were dialysed against ten times the volume of normal medium for 72 h, changing the dialysate on four occasions ●, Dialysed supernatant, ○, untreated supernatant

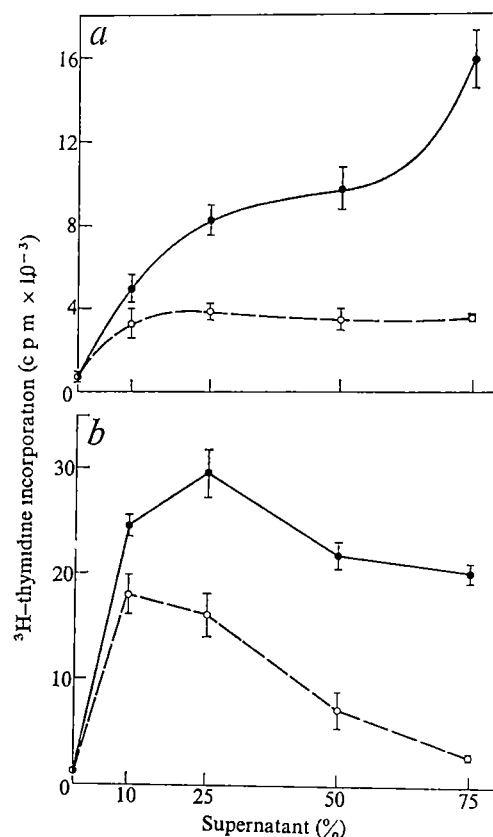


Fig. 3 Response of thymocytes to supernatants of peritoneal exudate cells For explanation see text a, No PHA, b, with PHA Symbols are as in Fig 2

(± 109) In agreement with the data of Gery *et al.*⁴, the untreated culture supernatant increased the PHA response of thymocytes up to $18,253 \pm 1,962$, at 10% (v/v) concentration This culture supernatant inhibited at high concentrations (more than 10%) The dialysed supernatant, on the other hand, was much more potent and showed, at the most, a small decline at high concentrations

In other experiments we have dissociated the inhibitory and stimulatory activities by varying the density of the peritoneal exudate cells All results described before were obtained with culture fluids obtained from 10^7 cells per ml plated in 35-mm plastic dishes A cell density of 2×10^6 per ml produced supernatants in which inhibitory activity was decreased in proportion to the reduction in cell density but in which stimulatory activity was maintained at about the same intensity

An inhibitor or a stimulatory factor for cell proliferation released from peritoneal exudate cells explains some of the divergent results previously published, but raises important points concerning their chemical nature and biological significance All evidence points to the macrophage as the source of both materials since peritoneal exudate cells devoid of lymphocytes released the same amount of activity (ref 1 and unpublished observations) The relationship between the state of maturation and/or activation of the macrophages and the two factors, however, are not clear It is possible that in a given situation inhibition or stimulation could result from such conditions as number of macrophages, anatomical relationship between macrophages and the target cell, the state of the macrophage and the nature of the target cell in question The two factors must now be considered with regards to the well known role of macrophages in immune induction⁵ and in cellular type of immunities⁶

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Chromosomal variation and the establishment of somatic cell lines *in vitro*

WHEN somatic cells from a number of species are explanted *in vitro*, they may either completely fail to grow, or they may continue to divide for a limited number of generations, at a progressively lower rate and with decreasing plating efficiency. They will eventually die unless, from this "crisis", a new population emerges with altered properties (see for example ref. 1). This new population, the established line, is usually capable of existing indefinitely and its growth rate and plating efficiency progressively increase until they stabilise at a level characteristic of each individual line.

The chromosomal constitution of these established lines is virtually always different from that of the parental primary cells and it is still a matter of debate whether these karyotype changes precede or follow establishment.

A parasexual cycle of polyploidisation-segregation has been found in human fibroblasts before crisis² and together with the fact that senescent human fibroblasts can be rescued by cell fusion³—which is normally followed by chromosome segregation⁴—suggests that polyploidisation may be important for establishment, perhaps as a source of variability. As the number of centromeres is subject to certain restrictions⁵ and multipolar mitoses are frequent⁶ the polyploid fraction would be unstable and would tend to generate a whole range of new types, often pseudo or quasi-diploid. Such variations in gene balance would give greater opportunities for generating types capable of existing indefinitely. Therefore polyploidisation, no matter how induced, could promote the growth of primary cells explanted *in vitro*. To test this hypothesis, we used various treatments which ultimately induce polyploidisation to determine whether the treated cell populations showed a better adaptation to *in vitro* conditions.

Whole mouse embryo cells, 4 d after explantation, were treated with β -propiolactone inactivated Sendai virus to promote cell fusion and then seeded in agar. Four colonies developed from a total of 5×10^6 Sendai-treated cells whereas no colonies developed from 5×10^7 untreated control cells. This growth could not be caused by growth-promoting factors present in the virus suspension since the virus had no effect on sparse cells in monolayers. Moreover, cell fusion rather than simple cell aggregation seems to be responsible because the colonies grown in agar, when isolated, propagated in monolayer and replated in agar, showed a plating efficiency of approximately 10^{-4} .

Chick and hamster cells were also treated with polyploidising agents such as griseofulvin, cytochalasin B or inactivated Sendai virus. Whole embryo primary cells, 4 d after explantation, were trypsinised and either treated with inactivated Sendai virus (200 haemagglutinating units per ml for 30 min) and then plated at 1.5×10^4 cells cm^{-2} , or plated

at the same concentration and treated the next day with griseofulvin ($50 \mu\text{g ml}^{-1}$) or cytochalasin B (1 and $5 \mu\text{g ml}^{-1}$). (In the case of chick cells, the plating concentration was 10^4 cm^{-2} .) Ten hours later the drug was removed. After 3 d all dishes were trypsinised and the cells plated at 500 cm^{-2} (1,000 in the case of chick cells).

At this cell density the control cells did not grow whereas colonies developed in the other dishes. Some of these colonies were isolated and propagated in monolayer. They gave rise to established lines with a success rate greater than 0.3 in the case of mouse and 0.12 in the case of hamster. With chick fibroblasts no permanent lines were obtained but the proliferation time was extended (85 d in griseofulvin-treated cells compared with 40 d for the control).

The growth-promoting effect can also be measured in terms of mitotic index (Table 1). A few days after treatment the mitotic index increased but there was a concurrent decrease in the polyploid fraction. This indicated that the proliferating fraction might not be represented by polyploid cells but by some of their segregation products.

Table 1 Mitotic index and frequency of polyploids

Time (d)	Control		Cytochalasin treated	
	Mitoses (%)	Polyploid (%)	Mitoses (%)	Polyploid (%)
1	8.3	2.3	4.0	25.0
2	4.7	8.1	3.2	23.2
4	1.8	6.2	1.8	23.2
5	1.9	7.3	4.6	14.7
6	1.6	5.9	5.2	13.0

Mitotic index and frequency of polyploids (cells with more than 60 chromosomes) in whole embryo mouse cells treated with cytochalasin B. Primary mouse cells were trypsinised 4 d after explantation and seeded at 1.5×10^4 cells cm^{-2} . Next day cytochalasin B was added at $1 \mu\text{g ml}^{-1}$ and removed 18 h later (time zero). Each subsequent day coverslips were set up with 2×10^4 cells cm^{-2} . Next day colchicine was added and 3 h later the coverslips were stained.

This possibility was examined with cells from *Mesocricetus auratus* which are generally difficult to establish and tend to be pseudo-diploid and whose chromosomes are easily identified⁹⁻¹¹. We observed a progressive loss of viability *in vitro* with these cells. In six generations the plating efficiency dropped from 0.52 to less than 0.001 and the growth rate, initially 1.5 d^{-1} fell to 0.4 d^{-1} . This only occurred however, if the cell density was kept above 5×10^3 cells cm^{-2} , at lower cell densities, untreated cells did not grow, whereas cells treated with any one of the polyploidising agents gave rise to colonies, 13 of which were isolated and developed into established lines. But when we seeded treated or untreated cells at densities ranging from 5×10^3 to 5×10^4 cells cm^{-2} and subsequently passaged the confluent dish that had the smallest inoculum, we succeeded in establishing lines from these mass cultures. The properties of these lines, 7 months after continuous cultivation, were indistinguishable from those of the 13 lines obtained after polyploidisation in respect of plating efficiency, which varied between 0.05 and 0.40, and growth rates, which were between 0.8 and 1.5 d^{-1} . When tested 5 months after explantation, two treated and one control line gave palpable tumours in less than 1 month when 3-month-old animals were injected subcutaneously with no fewer than 10^5 cells.

The chromosomal constitution of all these lines was followed from the first passage after explantation for 7 months. The number of polyploid mitoses varied initially from zero (primaries, untreated) to 40% (cells treated with $5 \mu\text{g ml}^{-1}$ cytochalasin B). The difference between treated and untreated cultures decreased however, and by the third week clear differences were no longer observed. In most of the 130 preparations examined, the percentage of polyploids was between 5 and 25. Quasi-diploid cells were in the majority but a stem line could not be identified¹².

When chromosomes were analysed for their banding pattern no real diploid metaphases could be found after the third week in all cases. The most common departures from normality were trisomies and monosomies and the number of translocations and other detectable structural aberrations, at up to five months, was usually no more than 2 or 3 per metaphase.

The conclusion we can draw from this set of experiments is that the karyotypal heterogeneity, known to be common in established lines¹³ begins soon after explantation, much sooner than suggested by earlier studies^{1,9,10}. Polyploidisation and the successive appearance of pseudo and quasi-diploid cells plays an important role in the evolution leading to establishment. This type of karyotype evolution may indeed be a rather general phenomenon since similar steps have been described *in vivo* during liver regeneration and in the bone marrow of adult and ageing hamsters^{12,14,15}.

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H₁ and H₂ receptors in the histamine-induced accumulation of cyclic AMP in guinea pig brain slices

EVIDENCE has accumulated suggesting that several transmitters, as well as hormonal substances, mediate their actions on effector cells by an increase in the intracellular level of adenosine cyclic 3',5'-monophosphate (cyclic AMP).

Like other biogenic amines, histamine is able to stimulate the cyclic AMP-generating system in brain slices of different animal species². Moreover, this action of histamine on brain cells, like other cerebral actions (either electrophysiological³ or behavioural⁴), seems to be mediated by means of an interaction with specific receptors, as it is not blocked by adrenergic antagonists but prevented by classical antihistamines⁵. In peripheral tissues two classes of histamine receptors seem to be present as evidenced by specific antagonists or agonists. Ash and Schild⁶ have termed H₁ those blocked by classical antihistamines and H₂ the others, responsible, for example, for the gastric secretory effect of the amine. Since the discovery of specific antagonists and agonists of these H₂-receptors⁷, the existence of an H₂-mediated stimulation of adenylyl cyclase has been reported, as in fundic gastric mucosa of guinea pig⁸. We report the presence in brain of both classes of receptors involved in the histamine-induced accumulation of cyclic AMP.

Cyclic AMP accumulation was studied in an *in vitro* model, derived from that described⁹. Male Hartley guinea pigs (300 g) were killed by decapitation, their brain removed and slices of cerebral cortical gray matter (250 μ m thick)

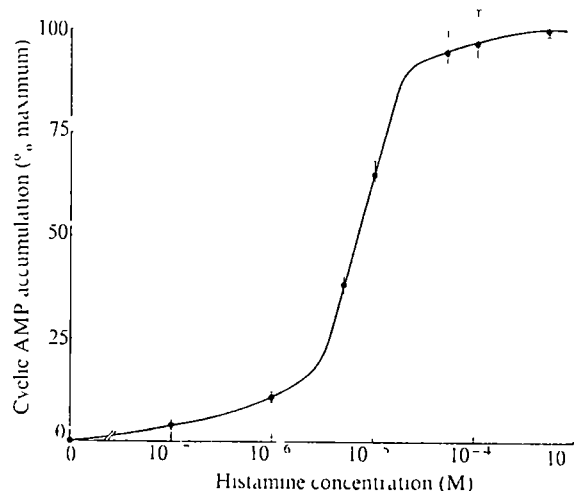


Fig 1 Effect of histamine on cyclic AMP accumulation in guinea pig cortical slices. In the absence of added histamine 5.3 ± 0.3 pmol per mg protein of cyclic AMP were accumulated. The percentage of maximal accumulation of cyclic AMP, which represents $381 \pm 6\%$ of the basal level, is plotted as a function of histamine concentration. Mean \pm s.e.m. of five samples.

were rapidly prepared with a McIlwain tissue slicer. The slices were preincubated at 37°C for 30 min in the presence of 4 μ M adenine in a Krebs-Ringer bicarbonate medium (about 50 ml per g of slices), under a constant stream of a mixture of O₂:CO₂ (95:5) in a Dubnoff metabolic shaker. The slices were then pooled, washed twice with fresh medium and distributed in incubation tubes, about 7.5 mg of tissue per tube. After the addition of test substances, the tubes were gassed and capped, the incubations were continued at 37°C and terminated by homogenisation of the slices with a sonicator. Homogenates were heated at 95°C for 8 min, after centrifugation, the amount of cyclic AMP formed in the slices was measured in 10 μ l aliquots of the supernatant by the protein kinase binding method⁹. All test agents did not compete for the binding of cyclic AMP and the specificity of the assay was tested by incubation of selected samples with purified beef heart phosphodiesterase. Proteins were determined by the method of Lowry *et al.*¹⁰ with bovine serum albumin as standard.

Preliminary experiments indicated that the level of cyclic AMP in control slices was constant within at least 15 min of incubation, that the accumulation of cyclic AMP induced by 100 μ M histamine reached its maximal value after 10 min and that it remained at this level until 15 min. In further experiments, incubations were therefore continued for 10 min after the addition of the different test substances. Figure 1 shows the increased accumulation of cyclic AMP in cortical slices as a function of histamine concentration, the maximal effect was achieved by 50 μ M histamine and the half-maximal stimulation was obtained with 7 μ M histamine.

Two specific histamine antagonists were used to assess the characteristics of the cerebral receptors involved in the accumulation of cyclic AMP: mepyramine, a classical anti-histamine, which antagonises the activation of the H₁-receptors⁶, and metiamide, a new antagonist of the H₂-mediated histamine response¹¹. Figure 2 shows the effect of increasing concentrations of mepyramine or metiamide on the maximal accumulation of cyclic AMP induced by 50 μ M histamine. Both these agents were able to block the effect of histamine in a dose-dependent manner, although to a limited extent, that is, about 50%, in each case. The ID₅₀ (concentration of the drug giving 50% of its maximal inhibition) of mepyramine and metiamide were 0.06 μ M and 6 μ M, respectively, and the maximal antagonism was achieved by 1 μ M and 100 μ M.

Table 1 Effects of various effectors on cyclic AMP (pmol per mg protein) accumulation in guinea pig cortex slices

Agonist	Antagonist	Metiamide (100 μ M)	Mepyramine (1 μ M) + metiamide (100 μ M)
None	None	4 88 \pm 0 46*	4 58 \pm 0 26*
Histamine (50 μ M)	None	6 44 \pm 0 25†	
4-Methylhistamine (10 μ M)	Mepyramine (1 μ M)		
	(50 μ M)		
	(100 μ M)	4 99 \pm 0 29*	

* Not significantly different from controls

† Significantly different from controls, $P < 0.05$ Mean \pm s.e.m. of 3–8 experiments

Moreover, when the two antagonists were added together, the histamine-induced stimulation was completely abolished (Table 1). The effect of 4-methylhistamine, a H_2 -agonist devoid of any significant H_1 -mediated action⁷, was evaluated to further characterise the cerebral histamine receptors. 4-Methylhistamine stimulated the cyclic AMP accumulation in a dose-dependent manner, the response was not affected by mepyramine (1 μ M) but was completely abolished by metiamide (100 μ M) (Table 1).

Our results agree well with previous reports on histamine stimulation of cyclic AMP synthesis in the guinea pig cortex^{2,5,12}. The partial blockade of the response of histamine caused by mepyramine, a classical H_1 -antagonist, and metiamide, a new H_2 -antagonist, clearly suggests the involvement of both H_1 - and H_2 -receptors in the effect of histamine on the cyclic AMP generating system. Chasin *et al.*¹² have found that classical antihistamines were not able to block completely the effect of histamine. Further support for the presence of H_2 -receptors is provided by the fact that 4-methylhistamine, a specific H_2 -agonist, also stimulates cyclic AMP synthesis, an effect blocked by a H_2 -antagonist and not modified by a H_1 -antagonist. Moreover, the additive effects of the two antagonists suggest that the activation of one class of receptor does not affect the activation of the other.

The apparent 'dissociation constant' of histamine for the complex 'receptor-cyclic AMP generating system' is about 7 μ M and does not differ from that reported¹³ for human astrocytoma cells¹³, however, it is far higher than the dissociation constants of histamine for H_1 - and H_2 -receptors determined in tissues containing an homogeneous population

of each receptor (0.09 μ M (ref. 14) and 0.6 μ M (ref. 7), respectively). On the other hand, the ratio of ID_{50} of mepyramine and metiamide is of the same order of magnitude as the ratio of their dissociation constant in peripheral tissues^{6,11}. Therefore, our results suggest that the H_1 - and H_2 -receptors in the brain present common characteristics with those involved in the various actions of histamine in peripheral organs. The fact that we measured the histamine-induced cyclic AMP accumulation in slices, that is, the net result of a complex sequence of interactions, could explain the discrepancies in the dissociation constants for histamine.

In peripheral tissues containing the two classes, H_1 - and H_2 -receptors are often associated with dual effects of histamine, as in the superior cervical ganglion¹⁵. It is tempting, therefore, to speculate that in brain also, the increased intracellular level of cyclic AMP induced by histamine mediates dual effects according to the type of receptor activated by the amine. This could be the case if the different receptors are localised on different classes of brain cells. Consistent with this view is the histamine-induced accumulation of cyclic AMP in human astrocytoma cells¹³, suggesting the presence of histamine receptors on glial cells, whereas the same action on rat cortex synaptosomes¹⁶ suggests the presence of histamine receptors on nerve cells. On the other hand, the possibility that the same cerebral cell possesses the two types of receptors cannot be excluded, in this case the dual responses of the cell to histamine could reflect a localisation on different parts of the membrane and/or the triggering of cellular mechanisms which may occur with different time scales. In other respects, that at least one of the two types of receptors may be localised at cortical synapses is suggested by the recently demonstrated existence of an ascending histaminergic pathway in the medial forebrain bundle projecting into the whole telencephalon^{17,18}.

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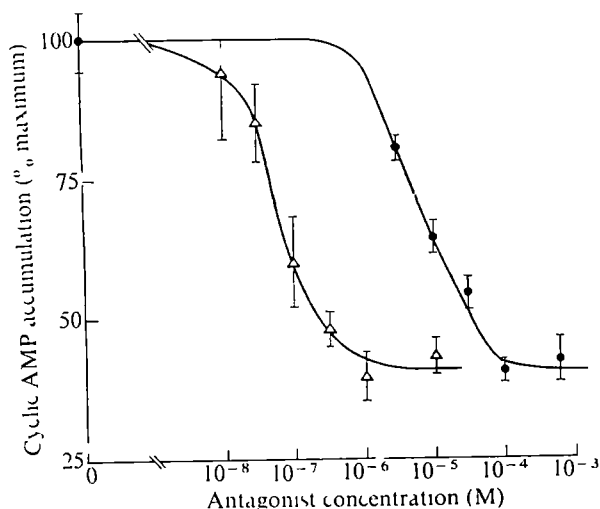
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Fig. 2 Effect of mepyramine (Δ) and metiamide (\bullet) on the maximal accumulation of cyclic AMP induced by histamine. The percentage of the cyclic AMP accumulation induced by 50 μ M histamine is plotted as a function of the concentration of each antagonist. The basal level of cyclic AMP in the absence of histamine (4.4 ± 0.4 pmol per mg protein) was not modified by the antagonists at any concentration. Mean \pm s.e.m. of four samples.



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Experimental allergic encephalomyelitis: dissociation of neurological symptoms from lipid alterations in brain

EXPERIMENTAL allergic encephalomyelitis (EAE) is an autoimmune, paralytic disease that can be induced in experimental animals by injection of whole tissue from the central nervous system (CNS), or the purified myelin basic protein (PBP) homogenised in Freund's adjuvant. In some respects, it resembles certain human demyelinating diseases¹⁻³. Preferential changes occur in the metabolism of glycosphingolipids in the CNS³⁻⁶, some of which do not seem to be directly related to the presence of clinical symptoms⁴. In the guinea pig, changes in some of the myelin components seem to depend on the disease-inducing substance used. The cerebroside content of the CNS changes when this animal is sensitised with whole CNS, or with PBP and CNS lipids, but not when PBP alone is used as encephalitogen. The content of sulphatides changes the extent depending on the composition of the encephalitogenic preparation injected⁶. We report here that changes in the level of glycosphingolipids may arise in response to components other than the myelin basic protein and without the simultaneous occurrence of paralytic symptoms.

We have studied the brain lipid content of guinea pigs injected with mixtures of CNS lipids and non-encephalitogenic proteins. The latter preparations were chosen to include basic or non-basic proteins mixed or not with CNS lipids. There are some indications that the susceptibility to different CNS antigens may vary with the strain of animals used and this, in turn, may influence the magnitude of lipid alterations in EAE^{3,5,6}. As mixed bred animals from different sources were used in our studies, controls injected with encephalitogenic preparations were included to compare the lipid alterations found in the EAE animals with those obtained previously⁶.

Forty random bred, adult guinea pigs, of mixed colour and 500-900 g body weight, were randomly distributed in eight

groups and injected intradermally in each hind foot as in Table 1. The amounts of each lipid and PBP injected to animals of groups BP and BPL corresponded to the quantities of these components normally present in the amount of frozen rat spinal cord administered to group CNS. The quantity of PBP used is well in excess of the amount required to produce a high incidence of acute EAE in guinea pigs⁷. Thirteen days after injection the characteristic symptoms were present in all the animals injected with the encephalitogenic preparations (groups CNS, BP and BPL) whereas no neurological effects could be seen in the guinea pigs from groups CF, Ab, Py, AbL and PyL. All of the animals were killed by decapitation 14 days after the injection, the brain quickly removed and its lipid content analysed. Details of the preparation of PBP and CNS lipids and quantitative determinations employed were described previously⁶.

Table 1 shows that the differences in the contents of cerebroside, sulphatides and gangliosides in animals with paralytic symptoms were in general similar to those recently reported for groups CNS, BP and BPL (ref. 6). The changes for gangliosides were again not very significant. We confirmed our previous observations that the injection of PBP (group BP) induced a decrease in the content of sulphatides but not in cerebroside and that the inclusion of lipids in the injection mixture (group BPL) is necessary to induce alterations in the cerebroside levels and to lower significantly the sulphatide content⁶. The animals from groups Ab, Py, AbL and PyL did not differ in physical appearance from the controls (group CF), nor did albumin-injected animals (group Ab) differ significantly in any of the lipids studied. The injection of the basic protein poly-L-lysine (group Py) produced a pattern of alterations closely resembling that found in animals injected with PBP. The importance of lipids in the injection mixture for inducing the changes without the need for encephalitogenic protein and without the concomitant appearance of neurological effects is clearly indicated in groups AbL and PyL. In these groups the changes were very similar to those observed in animals from groups BPL and CNS. The whole mixture of lipids or only particular components could be necessary.

Since the method used for the determination of sulphatide may be altered by other lipids, and values for cerebroside have been obtained by difference⁶, a statistical comparison was made of the total hexose content of the monohexosyl ceramide fractions (cerebroside plus sulphatide). The results obtained

Table 1 Lipid content of the brain in EAE and non-EAE guinea pigs

Animals	Total lipids (mg per g wet weight)	Sulphatides (mg %)	Cerebroside (mg %)	Gangliosides (μ mol sialic acid %)	Total cholesterol (mg %)
Group CF*	81.94 ± 3.53	5.76 ± 0.25	14.86 ± 0.94	2.08 ± 0.13	19.71 ± 0.45
Percentage differences with respect to group CF					
EAE					
Group CNS	1.7	-54.7%	-45.9%	-23.6%	0.5
Group BP	6.0	-31.3%	-1.3	-20.7%	0.1
Group BPL	2.6	-50.7%	-52.2%	-18.8%	4.4
Non-EAE					
Group Ab	2.7	-17.0	4.8	1.9	1.9
Group AbL	6.0	-53.3%	-32.6%	-14.9	-3.6
Group Py	1.9	-42.5%	1.3	-16.3%	5.6
Group PyL	6.2	-66.3%	-25.4%	-22.1%	-1.1

Each group comprised five animals and the results are mean values \pm s.e.m. Animals were injected intradermally in each hind foot according to the following scheme: Group CF, 0.25 ml Freund's complete adjuvant (Difco Laboratories, Detroit, Michigan) emulsified with 10% water. Group CNS, the same as CF but including 35 mg of homogenised adult rat spinal cord. Groups BP, Ab and Py, the same as CF but including, respectively, 0.21 mg PBP from rat spinal cord, 0.21 mg bovine serum albumin (crystallised and lyophilised, Sigma, USA) or 0.21 mg poly-L-lysine (hydrobromide, Type I, molecular weight 41,000, Sigma, USA). Groups BPL, AbL and PyL, the same as groups BP, Ab or Py, respectively, plus a mixture of CNS lipids consisting of 0.16 mg sulphatides, 0.7 mg cerebroside, 0.85 mg cholesterol, gangliosides corresponding to 0.02 μ mol sialic acid, and phospholipids corresponding to 2.5 μ mol lipid phosphorus. Each of the preparations was sonicated for 3 min at 20 kHz in a Branson sonifier before injection.

*Individual lipids in group CF were expressed per 100 mg total lipids. In all the other groups the figures represent percentage differences between means, taking the values for group CF as 100%. P was calculated by Student's *t* test for non-correlated samples.

†Significant at $P < 0.02$

‡Significant at $P < 0.05$

§Significant at $P < 0.001$

in the different groups were consistent with the changes shown in Table 1. Thus, we found decreases of 48.4% ($P < 0.001$) in group CNS, 9.8% (not significant) in group BP, 51.8% ($P < 0.001$) in group BPL, 0.7% (not significant) in group Ab, 38.4% ($P < 0.02$) in group AbL, 11.1% (not significant) in group Py and 37.1% ($P < 0.02$) in group PyL, (control value 25.0 ± 1.2 μ mol glycolipid hexose per 100 mg of total lipid).

Our observations indicate that the presence of PBP in the injection mixture, necessary to induce the neurological disturbances observed in EAE, is not specifically required to induce the lipid alterations in the CNS. On this basis, EAE classically induced with whole CNS seems to be a composite result of different effects: the phenomena related to the paralytic symptoms elicited by the PBP, and the alterations induced by the lipidic components present in the CNS tissue, independent of clinical symptoms. Such differences between EAE induced by whole CNS and by PBP have already been observed. A demyelinating, or myelination inhibition, factor is present in serum of EAE animals sensitised with whole CNS but it is not consistently induced when PBP is used as encephalitogen^{8,9}. The antibody to cerebroside is responsible both for the disturbances in the sulphatide metabolism and the demyelination effects observed in cord tissue cultures exposed to EAE serum⁸. Thus, it seems likely that our observations *in vivo* may be caused by similar phenomena, as suggested⁶. Inoculation of rabbits with cerebroside, adjuvant and albumin resulted in perivascular infiltration in the spinal cord but not paralysis¹⁰; these findings seem to be closely related to those recently observed in EAE.

On the other hand, the surprising results obtained with poly-L-lysine suggest that the basic nature of the protein injected is closely related to changes in the content of anionic lipids. This should lead to a study of the effects of the injection of other basic, acidic and neutral proteins on the lipid composition of the CNS. Recent findings¹¹⁻¹⁴ regarding the highly specific interaction of the myelin basic protein molecule with negatively charged lipids such as sulphatides at the air-water interface are relevant. If such interactions and molecular arrangements are also occurring *in vivo* in the CNS, the alterations observed may be interpreted as the consequence of specific disruptive responses directed towards some of the interacting molecules or particular structural assemblies.

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Marijuana, absinthe and the central nervous system

THERE are striking similarities between the psychological actions of the liqueur absinthe¹ and the experiences frequently reported by users of marijuana². We have therefore compared the properties of thujone and tetrahydrocannabinol (THC), which are believed to be the active principles of *Artemisia absinthium* and *Cannabis sativa*, respectively. Both substances are terpenoid, derived from the essential oils absinthol and cannabiol, and are formed by similar biosynthetic mechanisms^{3,4}.

The effects of absinthe have been known since the last century, but thujone has been conspicuously absent from recent lists of psychotropic plant products. It has been traditionally grouped with two $C_{10}H_{16}O$ isomers, camphor and menthol⁵, and classified as a convulsant poison. The molecular geometry of these three compounds is so different, however, that it is difficult to believe that, at low doses, they interact specifically with the same pharmacological receptors. At large doses, it is always possible that they exert similar, less specific actions by virtue of common physicochemical properties.

Thujone and THC have similar molecular geometry and similar functional groups available for metabolism. This close geometrical resemblance is illustrated in Fig. 1, in which the bonds common to both molecules are drawn as bold lines. The similarities include the gem-dimethyl groups at C₈, the C₇ methyl groups, the bonds connecting carbons 8, 4, 3, 2 and 1 of THC and 8, 5, 4, 3 and 2 of thujone, the α hydrogen at C₄ in THC and the cyclopropyl 5-6 bond of thujone and the 4-5 bond in THC and the 1-5 bond in thujone. Finally, although there is

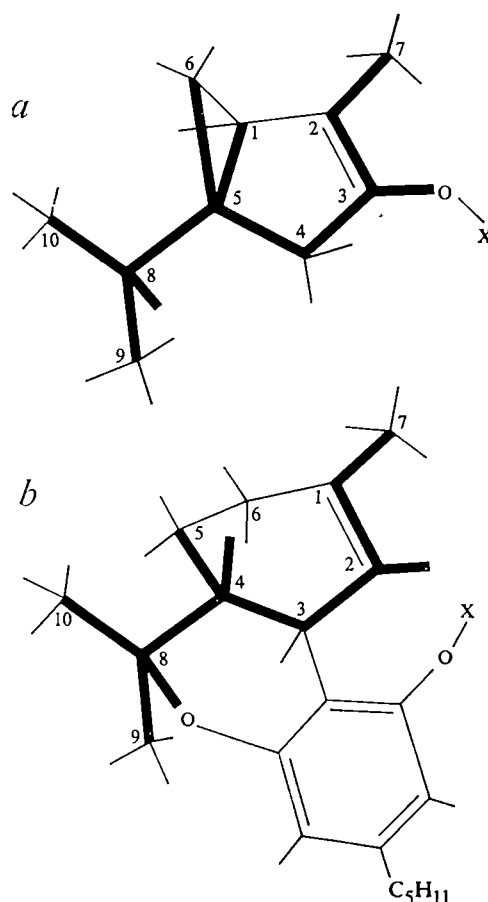


Fig. 1 Structural formulae of thujone-enol (a) and $\Delta^{1,6}$ -THC (b). Bonds common to both molecules are drawn as bold lines. X indicates the site of the receptor with which the oxygen of the thujone molecule or the hydroxyl group of the THC molecule may react. See text for details.

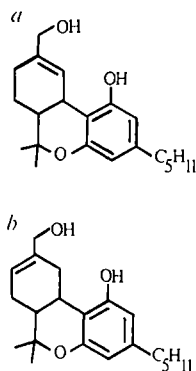


Fig 2 Products of metabolism of $\Delta^{1,2}$ -THC (a) and $\Delta^{1,6}$ -THC (b)

no direct correspondence between the oxygen of the thujone molecule and the hydroxyl group of THC, it seems possible that both react with a common site of a pharmacological receptor, such as that indicated by X in Fig 1, without changing the orientation or relative position of either molecule

These oxygen atoms are likely to be the principal pharmacological binding sites because the primary metabolites from $\Delta^{1,2}$ -THC (ref 6) and $\Delta^{1,6}$ -THC (ref 7), both of which are physiologically active, are products of oxidation at C_7 (Fig 2). Indeed, these metabolites have been suggested as the actual psychotomimetic agents in marijuana⁷

The $\Delta^{2,3}$ -enolic form of thujone contains an allylic group at $C_{3,2,7}$ which is analogous to $C_{2,1,7}$ in $\Delta^{1,2}$ -THC, both systems should be available for similar oxidative reactions. In fact the electron-releasing ability of oxygen in the thujone enol should increase the rate of hydrogen removal at C_7 relative to a comparable reaction in THC (ref 8). That thujone can exist in the $\Delta^{2,3}$ -enol form is shown by its reaction with permanganate, a process known to involve enols⁹. Since the reaction affords an almost quantitative yield of the keto-acid¹⁰ shown in Fig 3 there is little doubt that the $\Delta^{2,3}$ -enol form is involved. Further, the existence of enols in biological systems is well documented¹¹

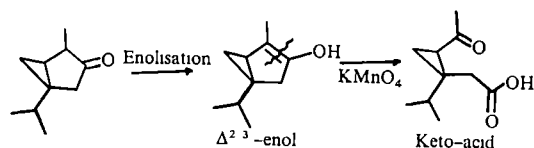


Fig 3 Permanganate oxidation of thujone by way of the $\Delta^{2,3}$ -enol

We propose therefore that both thujone and THC exert their psychotomimetic effects by interacting with a common receptor in the central nervous system. Topologically, this receptor should have a binding site for interaction with oxygen, a planar region to accommodate the allyl system, and pockets or cavities in which the alkyl and hydrogen substituents common to both drugs would fit. This hypothesis suggests new experimental approaches to study the pharmacology and toxicology of these and related compounds. A common mechanism of action for THC and thujone is also interesting from a historical and sociological point of view.

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Decrease in 17β -oestradiol receptor in brain of ageing rats

We have shown^{1,2} that the administration of 17β -oestradiol to ovariectomised female rats induces acetylcholinesterase (AChE, EC 3.1.1.7) in the cerebral hemisphere and cerebellum, and that this induction decreases with increasing age. The greatest induction occurs in the immature rat, and there is no induction in the old. As 17β -oestradiol receptors are present in rat brain^{3,4}, we suggested that the impairment of AChE induction in the brain of old rats may be due to a decrease in the level of this receptor. This study was designed to test our hypothesis, and the data show for the first time that it may be correct.

Immature (7 week), adult (44 week) and old (108 week) female Wistar rats maintained in the rat colony were used. The rats of the three age groups were bilaterally ovariectomised and reared on tap water and standard diet for 21 d to ensure complete disappearance of oestradiol from the blood as described earlier^{1,2}. They were killed on day 22 and the cerebral hemispheres were removed to prepare a 10% homogenate (w/v) in Krebs-Ringer bicarbonate buffer, pH 7.4, containing 2.8 mmol glucose.

The procedure followed for characterising the oestradiol-receptor complex of the cytosol was adopted from that of Wong and Burton⁵ for the hydrocortisone-receptor in the nuclei of placenta. 3H -oestradiol (0.2 μ Ci) (Radiochemical Centre, Amersham) was added to 1.0 ml aliquots of homogenate and the samples were kept on ice for 30 min with occasional stirring. They were then incubated at 37°C for 10 min in a water bath shaker after which 5.0 ml of 1.5×10^{-3} M $MgCl_2$ were added to each. Then they were kept in ice for 15 min and centrifuged at 700g for 15 min at 0°C. The pellet was resuspended in an equal volume of $MgCl_2$ and centrifuged as before. Both the supernatants were pooled and centrifuged at 14,500g for 1 h. The supernatant thus obtained was fractionated through a 11 \times 180 mm column of Sephadex G-25-80 (Sigma) at 2°C using an elution buffer of 0.6 M KCl, 10 mmol Tris and 1.5 mmol EDTA, pH 8.0. Fractions (1.0 ml) were collected and a 0.1 ml aliquot of each fraction was applied on 2 \times 2 mm Whatman No. 1 filter and dipped in 12.0 ml of scintillation fluid (PPO, POPOP, 4.04 in 10.1 toluene). The radioactivity was counted in a Packard Tri-carb liquid scintillation spectrometer (Model 3003). The concentration of protein (μ g ml⁻¹) was measured according to the method of Sutherland *et al.*⁶. The averages of the data collected from four or five rats of each age are given in Fig 1.

Figure 1 shows a sharp peak and a high level of oestradiol-protein complex in the cytosol of the immature rat as compared to those of the adult and the old rat. The peak for the radioactivity corresponds to the protein peak. It is also noteworthy that the peak for the labelled-complex gets diffused in the adult and the old indicating a loss of specificity or affinity of the receptor protein of the cerebral hemisphere which binds to oestradiol.

So far, the only enzyme of the cerebral hemisphere which

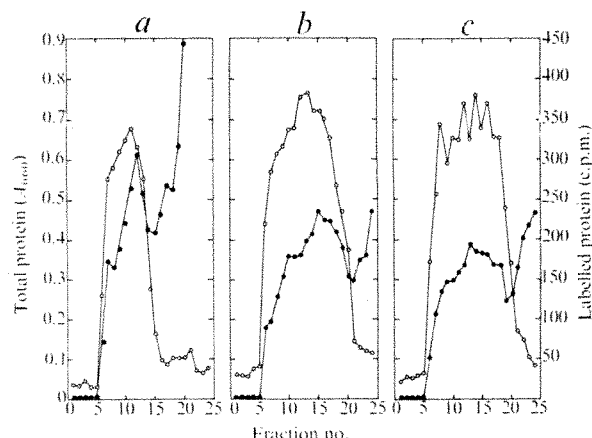


Fig. 1 Elution profile of labelled protein (^3H -oestradiol-protein complex) (●) and total protein (○) of the cytosol of the cerebral hemisphere of, a, 7; b, 44 and, c, 108-week-old ovariectomised rats by Sephadex G-25-80 chromatography.

is known to be induced by 17β -oestradiol is AChE. Our data do not show directly that it is this 17β -oestradiol-protein complex which mediates the induction of AChE but the positive correlation between the degree of induction of AChE and of the level of 17β -oestradiol-protein complex is of great significance.

The lag period for the induction of tyrosine aminotransferase in the liver of the rat is known to increase with age⁷. We have shown that the induction of malate dehydrogenase⁸ and glutamine synthetase⁹ of the liver of rats by cortisone is impaired in old age. This impairment is similar to that of AChE of the brain^{1,2}.

These data show that the impaired induction of certain enzymes by steroid hormones in old age may be due to the depletion of specific receptor proteins in the cytosol of target cells. The receptor being a protein, its synthesis may be under the control of a unique gene whose expression may be regulated by a specific factor (for example, a hormone) that decreases after development. Also, the decrease in the level of a specific receptor with high affinity, and the possibility of the appearance of certain proteins having un-specific affinity for the hormone may account for the diffused nature of the receptor peak in older rats. The possibility, however, of the appearance of molecules which may repress specific genes of these enzymes in old age cannot be ruled out. This is consistent with the view that programmed changes in the activities of genes begin after fertilisation and continue throughout the life span^{10,11}. This may account for the sequential changes in the enzyme pattern as observed by us for cytoplasmic alanine aminotransferase of the liver of the rat during its life span (M.S.K. and S.K.P., unpublished). This may, in turn, account for differentiation, development and senescence of an organism.

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Interdigitated repeated sequences in bovine satellite DNA

MOST eukaryote genomes contain some highly repeated DNA sequences. DNA hybridisation kinetic studies indicate that the smallest repeating unit of highly repetitious DNA is around 300 base pairs¹ but direct sequence analysis of highly repeated DNA has demonstrated a simple basic repeating unit of 4–12 nucleotides^{2–5}. It has been postulated that the repeated sequences have been generated by successive replications of some primary simple sequence^{1,6,7}. The discrepancy between sequence data and kinetic analysis may be explained by noting that the rate of reassociation is a function of length⁸; this data is consistent with a model of internal heterogeneity in the repeated, simple DNA sequence units^{9,10}. Although the hybridisation and sequence analyses have been successful in establishing certain features of reiterated DNA, these methods are not well suited to detect and study infrequent but regularly repeated DNA sequences interdigitated within the dominant short repeating units. The existence and nature of such interdigitated repeats are important to our understanding of the mechanism by which repeated DNA is generated. We have used site-specific endonucleases (restriction enzymes) to show that mutational events within simple sequence DNA may have been coupled with cycles of discrete replications.

If a stretch of DNA were comprised of only a simple repeated sequence which contains the restriction endonuclease recognition site, it would be cleaved to fragments the size of the simple sequence. Conversely, if the simple sequence lacked the recognition site, it would be refractory to digestion. The results of digesting bovine DNA with the sequence-specific endonuclease isolated from *Haemophilus influenzae* Rd (endo R·Hind II+III)^{11,12} and analysing the digestion products by polyacrylamide gel electrophoresis have already been reported^{13–16}. This enzyme produces an expected continuous distribution of DNA fragments; superimposed on this distribution are at least twelve distinct, specific fragments ranging in size from 1.2×10^5 to 2×10^6 , and reiteration frequencies from 2,000 to 140,000 (in a previous report¹⁴ the smallest fragment, XII, was assigned a molecular weight of 7.5×10^4 ; our more recent data indicate it is 1.2×10^5). These twelve size classes of fragments could only be produced if the fragment termini containing the restriction site occurred at regularly spaced repeated intervals.

To ascertain whether there are additional interdigitated repeats besides those detected by the *Hind* digestions, bovine DNA was hydrolysed with endo R·Hae (the restriction enzyme from *Haemophilus aegyptius*¹⁷). Approximately 30 discrete bands were produced (Fig. 1, third lane). These fragments were generally smaller than those observed in a *Hind* digestion (Fig. 1, first lane). Most of the low molecular weight *Hae* fragments (Fig. 1, region 27–33) increased in size progressively by steps of approximately 10 base pairs up to a fragment length of about 100 base pairs. This step increase is in the size range of the simple repeating sequences found in other organisms^{2–5}. Although the digestion profile in the region of the larger fragments becomes considerably more complex, it is tempting to extrapolate that incremental size increases are by some variable multiple of a 10 base repeat.

- The relationship between the two sets of fragments generated by *Hind* and *Hae* was determined. Figure 1 (first, second and third lanes) shows a comparison of the digestion profile produced by a *Hind* plus *Hae* digestion with the profiles produced by each enzyme alone. This comparison showed that all of the *Hind* fragments were abolished by *Hae*.

To determine whether the *Hae* fragments are contained within the *Hind* fragments, preparative quantities of several specific *Hind* fragments were eluted from gels¹² and analysed after secondary digestion with *Hae* (Fig. 1). One obvious aspect of these results is that the sum of the molecular weights of the *Hae* fragments approximately equals the molecular weight of their respective parental *Hind* fragments. But, in the case of *Hind* fragments V and VIII the molecular weights of the derived *Hae* fragments add up to twice that of their respective parents, suggesting that *Hind* V and VIII are each comprised of two fragments. Cross contamination between *Hind* fragments can be ruled out as an explanation for this observation, since control experiments in which *Hind* fragments were re-electrophoresed on gels, indicated this to be less than 10%. Similarly, the sum of the *Hae* fragments does not equal the molecular weight

of *Hind* fragment III unless *Hae* fragment 6 is added twice as suggested by its relative intensity. Thus, it is concluded that in each case the *Hae* fragments are comprised of the same nucleotide sequences as their respective *Hind* fragment.

A second point is that a number of the *Hae* bands are common to several different *Hind* fragments (in this sense 'common' refers to fragments with the same electrophoretic mobility). For example, many of the low molecular weight *Hae* fragments in the region 19 to 30 inclusive are common to *Hind* fragments IV, V, VI+VII, and VIII (Fig. 1). Similarly, *Hae* fragments 19 and 20 are common to *Hind* fragments II, III, V, VIII, and IX, and *Hae* fragment 16 is common to *Hind* fragments V, VI+VII, and VIII, and there are other cases. These instances of the same *Hae* fragment occurring within several different *Hind* fragments suggest considerable internal sequence similarity among some of the *Hind* fragments.

The juxtaposition of individual fragments can be discerned by identifying DNA regions containing a *Hind* site adjacent to a *Hae* site. Such a configuration will yield fragments not shared by the *Hind*, *Hae*, and combined *Hind* plus *Hae* digestion profiles. One such overlap has been identified in *Hind* fragment XII by virtue of fragments 24

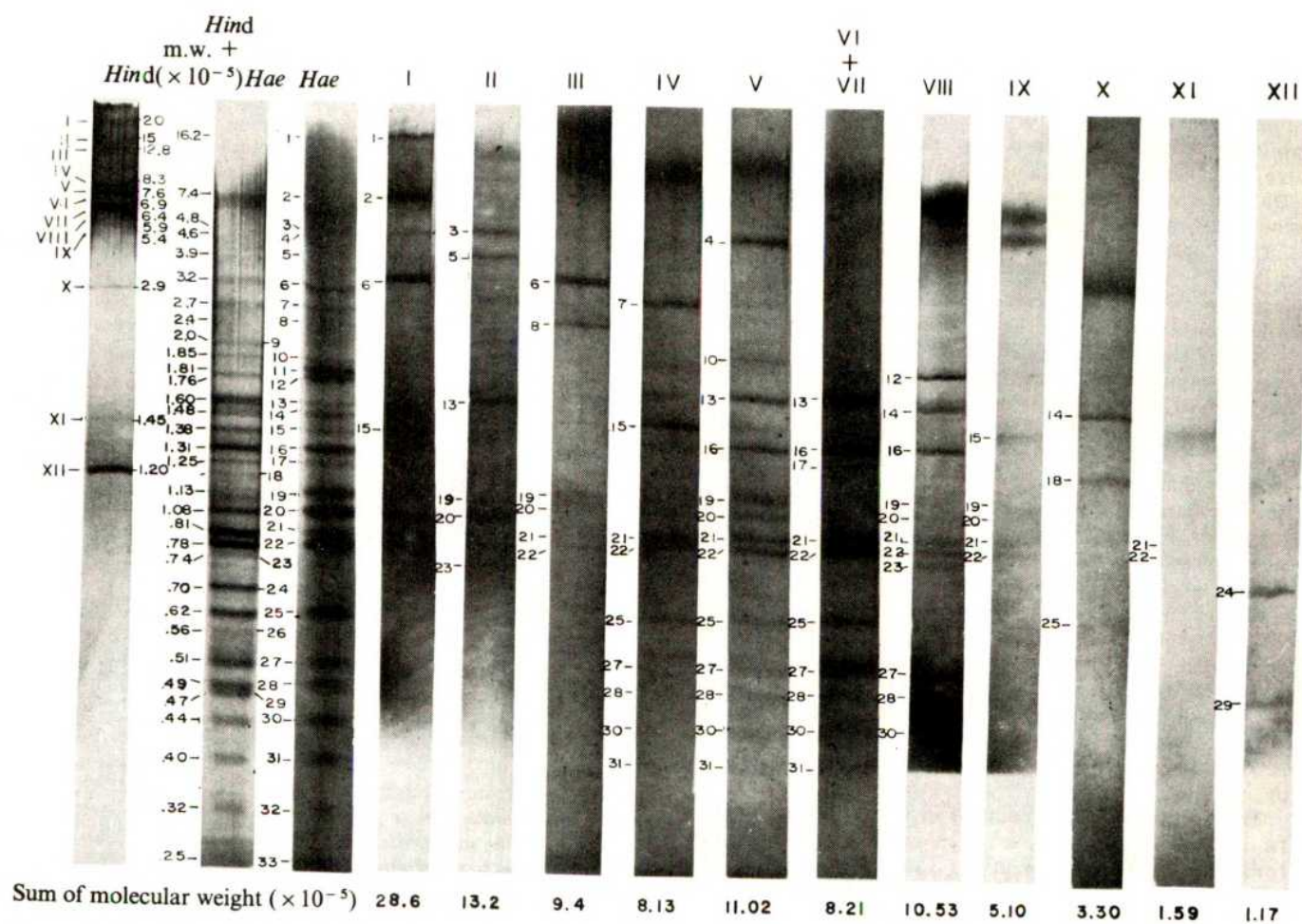


Fig. 1 *Hae* cleavage products of repeated *Hind* fragments in bovine DNA. The first three lanes are digests of total bovine DNA: first, *Hind* alone; second, *Hind*+*Hae*; third, *Hae* alone. Molecular weights were determined by coelectrophoresis with *Hind* fragments of $\Phi 80h$ DNA¹⁷. The remaining lanes are secondary digests prepared as follows. Total bovine DNA was hydrolysed with *Hind* and fractionated by electrophoresis on preparative 2% acrylamide-0.5% agarose slab gels at 4°C and 200 V (ref. 14). Individual bands were cut from the gel. The DNA was electrophoretically eluted and after concentration and dialysis was hydrolysed with *Hae*. Subsequent electrophoresis was on 4% acrylamide-0.5% agarose gels at 4°C and 200 V. The *Hind* fragments used as substrate for the secondary *Hae* digestion are given in Roman numerals at the top of each lane. Arabic numbers are assigned to the bands produced by hydrolysis with *Hind*+*Hae* or *Hae* alone. In some cases, faint *Hae* fragments occurring in *Hind* profiles are not labelled (for example, a *Hae* fragment in *Hind* fragment I corresponding to *Hae* fragment 3). There is a small amount ($\leq 10\%$) of cross contamination of *Hind* fragments in the preparative gels, and this is the most likely source of these faint bands. *Hind* fragments VI+VII have been presented together because they have not been adequately resolved on these gels. *Hind* fragment VII, however, is 15 times more abundant than *Hind* fragment VI¹¹; thus, *Hae* fragment 17 probably originates from *Hind* fragment VI.

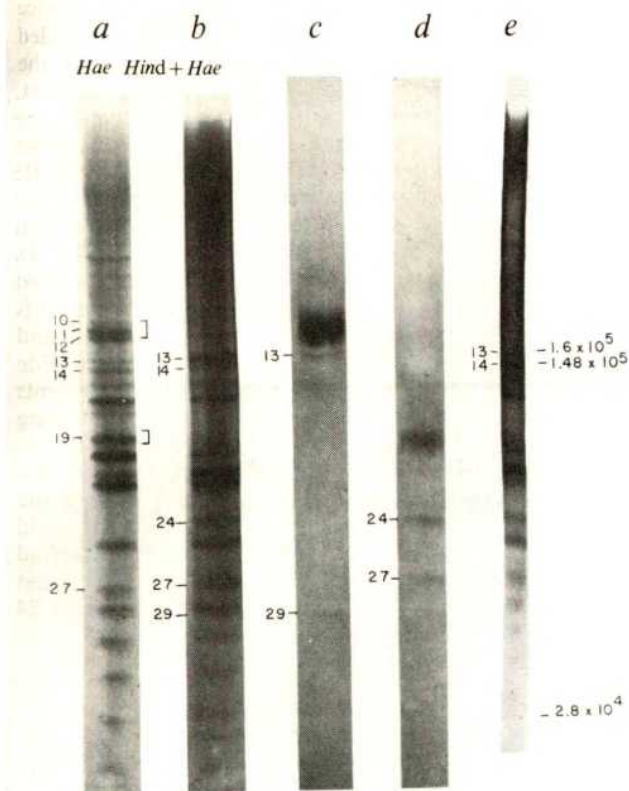


Fig. 2 Determination of the partial order of *Hae* fragments within and overlapping *Hind* fragments VII and XII. Bovine DNA (1 mg) was hydrolysed with *Hae* and electrophoresed on a 4% acrylamide-0.5% agarose slab gel. The regions of the gel indicated by brackets were cut out, and the DNA was eluted as described in Fig. 1. DNA from the upper bracketed region and from the lower bracketed region, containing *Hae* unique fragments 11 and 19, respectively (see text), were each further hydrolysed with *Hind* and electrophoresed in 4% acrylamide-0.5% agarose (lanes *c* and *d*, respectively). Bovine DNA hydrolysed with *Hae* (lane *a*) and *Hind*+*Hae* (lane *b*) were included as controls. The numbers conform to the band labelling system utilised in Fig. 1. Lane *e* is an *EcoRI* hydrolysate of *Hind*+*Hae* digested bovine DNA. The molecular weights of the products were determined as indicated in Fig. 1. See text and Fig. 1 for further discussion.

and 29 (Fig. 1, lane XII) which are present only in the combined *Hind* plus *Hae* digest. Nearest neighbour information between *Hind* fragment XII and other *Hind* fragments can be detected by identifying those *Hae* fragments which on *Hind* digestion give rise to 24 or 29 (Fig. 3). *Hae*

fragment 11, which is not found in the combined *Hind* plus *Hae* digest, when subsequently digested with *Hind* yields fragments 13 and 29, in addition to undigested material (Fig. 2, lane *c*). Fragment 13 is found in the *Hae* digests of *Hind* fragments II, V, and VI+VII. Of these potential *Hind* fragments as neighbours to fragments XII, only fragment VII seems likely. This deduction is based on earlier evidence in which we determined that the buoyant density of sheared DNA containing *Hind* XII was the same as that of sheared DNA containing *Hind* fragment VII, and was of a significantly different density than that of sheared DNAs containing the other *Hind* fragments as nearest neighbour candidates¹⁴.

The neighbour on the other side of *Hind* fragment XII can be determined by similar reasoning. *Hae* fragment 19 which is not found in the combined *Hind* plus *Hae* digest, when subsequently digested with *Hind* yields fragments 24 and 27 (Fig. 2, lane *d*). As noted above, fragment 24 is unique to *Hind* fragment XII. Fragment 27 is found in *Hind* fragments IV, V, VI+VII and VIII. From the buoyant density data described above, fragment VII is the most likely candidate as the neighbour to XII. From this it is concluded that fragments VII and XII occur adjacent to one another, in an alternating tandem fashion. Other cases of overlapping regions which can be discerned by comparing the *Hind*, *Hae*, and combined *Hind* plus *Hae* digestion profiles will not be discussed here since they are less well resolved.

This conclusion that *Hind* fragments VII and XII alternate with each other is consistent with the observation of Botchan¹⁶ that purified bovine satellite I DNA, when digested with *Hind*, gives rise only to products corresponding to our fragments VII and XII. We have observed that the endo *R-EcoRI* restriction site¹⁸ discussed in the work of Botchan in fact occurs in *Hae* fragment VII-13, because it disappears in a combined *Hind* plus *Hae* plus *EcoRI* digest and gives rise to fragments of molecular weights 0.3×10^5 and 1.48×10^5 (Fig. 2, lane *e*, and Fig. 3). Similar conclusions have also been reached by P. Philippsen (personal communication) with the further finding that in addition to fragments 24 and 29 there is a very small *Hae* fragment which is produced from the centre of *Hind* XII.

Having obtained these patterns with *Hind* and *Hae*, it was of interest to ascertain whether any other restriction enzymes yielded equally dominant repeat patterns. To test this, a series of experiments was carried out in collaboration with Dr R. J. Roberts. A *Hind* plus *Hae* digest of bovine DNA was subjected to digestions with each of ten additional restriction endonucleases which were provided by Dr Roberts: *Hpa* I and II, *Hha* I, and *Hph* I (ref. 19);

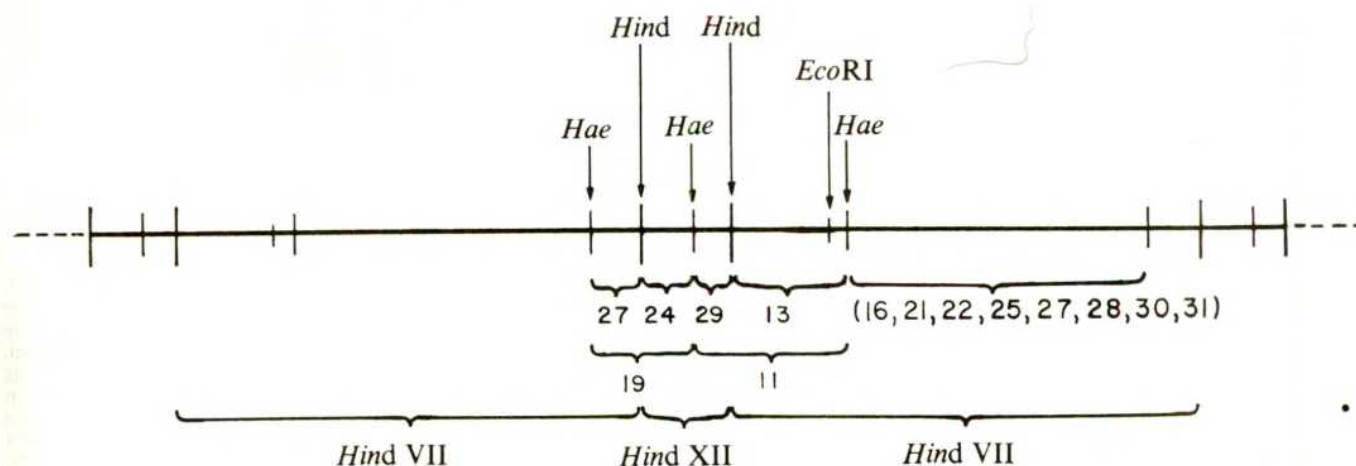
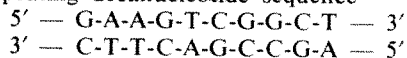


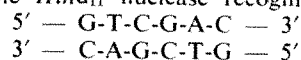
Fig. 3 Schematic representation of the arrangement of *Hind* fragments VII and XII and their internal *Hae* fragments. See the text and Figs 1 and 2 for further descriptions.

Alu I, isolated from *Arthrobacter luteus* (R. J. Roberts, unpublished); *Sma*, isolated from *Serratia marcescens* (C. Mulder, unpublished); *Hga* I, isolated from *H. gallinarum*²⁰; *Sal* I, isolated from *Streptomyces albus* G. (R. J. Roberts, unpublished); *Sac* I, isolated from *Streptomyces achromogenes* (R. J. Roberts, unpublished); and *Xan* I, isolated from *Xanthomonas amaranthicola* (R. J. Roberts, unpublished). The majority of the *Hind* plus *Hae* fragments were not abolished by these additional restriction enzymes, thus indicating that the repeats demonstrated by the rather frequent occurrence of the *Hae* cleavage site are quite sequence dependent (data not shown).

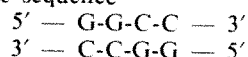
The internal DNA sequence within each of the fragments need not, *a priori*, contain reiterated DNA, but we have shown that most of the twelve *Hind* fragment size classes can be derived from positions in isopycnic gradients also known to contain satellite DNA (ref. 14). It has also been shown that at least two of the *Hind* fragments reanneal with the kinetics of a simple sequence¹⁶. Therefore, we postulate that the repeated *Hind* and *Hae* nuclease restriction sites differ only by one or a few bases from some primary repeating sequences. For example, in the hypothetical primary repeating decanucleotide sequence



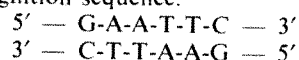
a single transition in the eighth position could yield one of the two possible *Hind*_{II} nuclease recognition sequences¹¹,



(All twelve *Hind* fragments except for fragment VIII are bounded by two *Hind*_{II} sites; unpublished results.) Similarly, a single transition in the tenth position of this decanucleotide yields the *Hae* sequence



(K. Murray and R. Roberts, personal communication). A transversion in position 4 of the decanucleotide would yield the *Eco*RI recognition sequence.



(ref. 18) which occurs in fragment 13 (see Fig. 3).

The data presented in this report demonstrate the existence of different orders of interdigitated repeats within the satellite fraction of DNA. These results support the idea, advanced previously by others^{1,6,7}, that multiple rounds of replication, and/or unequal crossing over are separated by intervening mutational events.

Sequence analyses now in progress should serve to establish the precise relationship between the *Hind* and *Hae* nuclease recognition sites and the postulated primary simple repeat.

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Super dense carotenoid spectra resolved in single cone oil droplets

CONE visual receptors in reptiles and birds contain brilliant yellow, orange or red oil droplets, 3-6 µm in diameter, through which light must pass before reaching the visual pigment. The possible role of these brightly coloured organelles in colour vision has been disputed¹⁻⁶. Their colours are thought to be caused by various carotenoids whose characteristically peaked absorption spectra in organic solvent extracts of whole retinas were first reported by Wald and Zussman⁷. In spite of this, subsequent workers have failed to identify the dramatic carotenoid spectra⁸ in individual oil droplets by direct methods of microspectrophotometry⁹⁻¹³. Instead, structureless cutoff absorbance curves reminiscent of commercial colour filters have been found to truncate at about one absorbance unit without any evidence of carotenoid spectral fine structure (Fig. 1).

It is not known why individual micromasurements do not substantiate the results of extraction. Neither is it understood, therefore, precisely how individual oil droplet colours are contrived. The flat-top and cutoff spectral character evident *in situ* might easily be accounted for by spectral overlap in various carotenoid mixtures. Alternatively, these properties might be a manifestation of instrumental limitations of microspectrophotometers¹⁴. Although our absorbance measurements on single oil droplets were done with some care, passing 1-2 µm diameter circular microbeams through their centres to reduce leakage of incident light around the sides, stray or scattered light might still dominate these measurements if the absorbance was very high¹⁴. Such artefacts could yield a plateau throughout the spectral region where true specimen density exceeds instrument capability.

To pursue this problem we initially improved our microspectrophotometer¹³ to record higher absorbances up to 2.7 but found the oil droplet spectra to plateau again at this new limit. Having achieved a practical limit for high-density microspectrophotometry, still without resolving spectral structure, we then turned to Lambert's law which suggests that absorbance might be reduced to measureably low values by reducing the optical pathlength. To accomplish this we squeezed oil droplets from freshly isolated retinas in a screw press under microscopic observation. We also air dried collections of oil droplets from distilled water to cause surface tension flattening. Still further thinning was achieved by exposure of air dried preparations to organic solvent vapours of hexane, pyridine, benzene or chloroform. We achieved only limited success with each method as we could only begin to see evidence of spectral fine structure. Moreover, the spectrum changed spontaneously on exposure to air and we could not achieve the quantitative results we now describe.

Retinas were removed from excised fresh turtle eyes (*Pseudemys scripta* or *Chelonia mydas*) in Ringer solution and were dropped into a small Petri dish of distilled water. After 30 min, a sediment of oil droplets released from lysed cells could be seen with a dissecting microscope. A drop of this sediment was sucked into a Pasteur pipette and transferred to a microscope

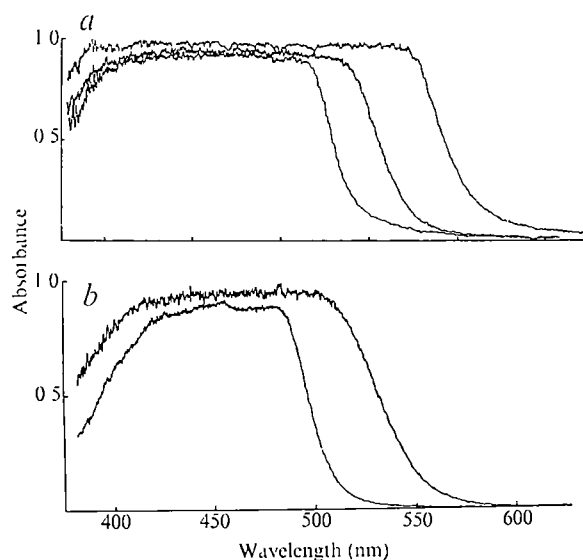


Fig 1 Spectra of individual cone oil droplets recorded directly in excised turtle retinas with a microspectrophotometer. Each spectrum shows a plateau near one absorbance unit. *a*, The swamp turtle, *Pseudemys*. From left to right, yellow, orange and red droplet spectra. *b*, The sea turtle, *Chelonia*. From left to right, yellow and orange droplet spectra.

slide. This was viewed at 650–1,000 \times magnification in an inverted microscope equipped with a Leitz micromanipulator. Individual oil droplets were then identified and their diameters measured. A 3–4 μ m tip micropipette attached to a pressure injection syringe was brought into contact with the oil droplet and a tiny drop of mineral oil (Squibb, USP C₈₋₁₂ hydrocarbon) expressed from the tip. As the two droplets fused, the pressure was quickly relieved and the pipette tip raised, leaving the new (20–50 μ m diameter) coalesced sphere attached to the glass slide with a swirling mixture of colour in its midst. Complete mixing, as evidenced by colour homogeneity, occurred within 10 s. The diameter of the new diluted droplet was measured and microspectrophotometry done using a 5 μ m microbeam passed through its centre. This produced resolved spectral structure in each case (Fig 2).

Using the conservation law, $C_1V_1 = C_2V_2$, and the Beer-Lambert law, $D = \epsilon cl$, where c is concentration, V is volume, D is optical density, ϵ is extinction coefficient, l is optical path-length and the subscripts 1 and 2 refer to the original and diluted droplet respectively, it is easy to see that the absorbance of pigment in the original droplet was

$$D_1 = D_2(R_2/R_1)^2 \quad (1)$$

if the droplets were spheres of radius R measured from their plan view. Histologically, the original droplets are spheres but as the diluted droplets are never seen in cross section, the above formula might not be applicable should the enlarged droplet flatten to become an ellipsoid or hemiellipsoid of revolution instead of a sphere. It can be shown, however, that these non-spherical forms yield self-cancelling effects on the dilution factor and pathlength leading to precisely the same formula as for spheres. Although it could be speculated that Beer's law might not apply at such high pigment concentration, we have evidence to the contrary, for the shape of the spectrum of the diluted pigment (Fig 2) at very long wavelength provides a satisfactory fit to the long wave part of the original spectrum (Fig 1) when scaled by the factor D_1/D_2 obtained from equation (1).

Thus, our experiments yield several conclusions (Fig 2, Table 1). Each pigment is exceedingly dense, indeed, such high recorded absorbances are unknown to us in any other cellular structure. The red and orange droplets of *Pseudemys* have the same spectral shape, that is, their pigments are identical. Their spectrum appears to match one of the extracts, that of astaxan-

thin^{7,8}, while that of the yellow droplet is similar to zeaxanthin or xanthophyll^{8,15}.

We conclude that in spite of similar composition the orange droplet cuts off at lower wavelength than the red simply because of its nearly tenfold lower concentration and optical density. The absorbance of the 6 μ m diameter red droplet, however, is 50–90 absorbance units. At peak, this droplet may transmit only 10^{-90} of light incident on it to the cone outer segment, that is, it cuts out 'completely' at peak. Preliminary results on the red droplet of birds also indicate extremely high optical density.

Table 1 Range of peak absorbance found in individual oil droplets by the method described in text

	R	O	Y
<i>Pseudemys</i>	50–90	6–9	12–18
<i>Chelonia</i>		4–7	3–4

R, red, O, orange, Y, yellow

Using its published extinction coefficient in hexane¹¹ it is possible to compute a concentration of about 1 M for astaxanthin in the *Pseudemys* red droplet. Using reasonable values for its partial molal volume, it can be concluded that astaxanthin itself occupies over half the space of the red oil droplet containing it. Indeed, if a red droplet is scratched with a micropipette, the material behaves like crayon or a viscous wax. Its failure to crystallise in these circumstances is probably consistent with the presence of a mixture of long chain *cis* and

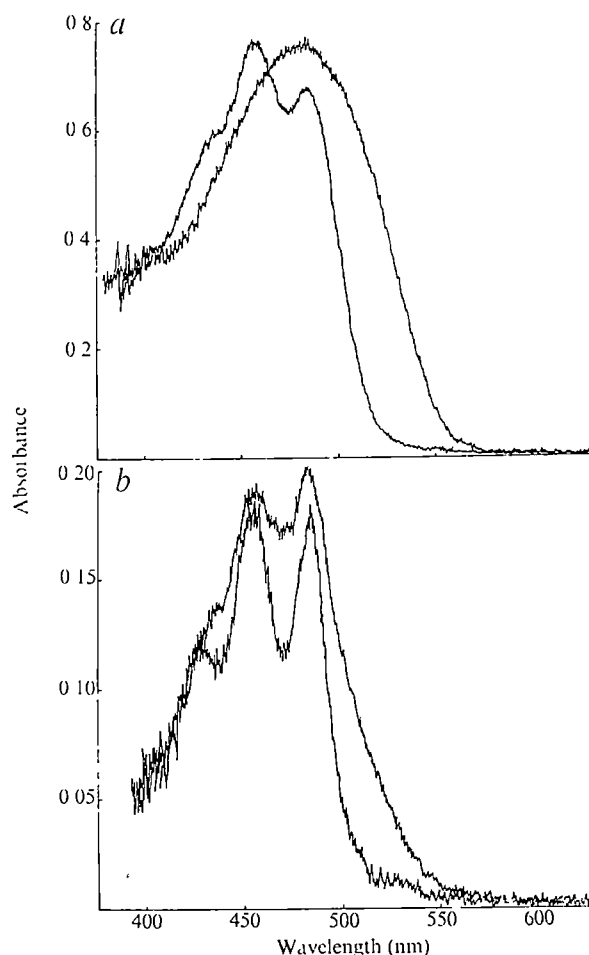


Fig 2 Spectra of individual turtle oil droplets after enlargement and dilution with mineral oil. *a*, *Pseudemys*, three-fingered spectrum of yellow droplet (left hand trace) singly peaked spectrum of orange and red droplets (right hand trace). *b*, *Chelonia*, sharply fingered spectrum of yellow droplet (lower trace), orange droplet (upper trace).

trans isomers that retain a hindered fluidity or domain structure. In *Chelonia* a somewhat different picture emerges. No red droplets are present but both orange and yellow diluted droplets have a three-fingered fine structure. They are not quite a match, however, and we can only say that each droplet seems to contain a single pigment of high absorbance, though not as imposing as those of *Pseudemys*.

The oil droplet pigments of both turtle species are sufficiently dense to strongly alter the spectral distribution of light striking the photosensitive visual pigments. They must therefore play a role in colour vision and like the visual pigments themselves¹⁶, only a single pigment species is present in each cell.

We thank Dr Leo Lipetz who suggested that coloured oil droplets might contain immeasurably dense pigments. This work was supported by grants from the United States National Eye Institute and the United States National Institute for Neurological Diseases and Stroke.

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Regulation of RNA polymerase II activity in a mutant rat myoblast cell line resistant to α amanitin

A MUTANT cell line, Ama102, resistant to the cytotoxic effects of α amanitin, the specific inhibitor of RNA polymerase II (refs 1 and 2), has been selected³ from cultures of Yaffe's⁴ rat skeletal muscle myoblast cell line, L₆, treated with mutagen. Ama102 cells have similar doubling times for growth in the absence or presence of 3 $\mu\text{g ml}^{-1}$ α amanitin whereas the Ama⁺ parent does not survive in this concentration of α amanitin. Studies *in vitro* (ref 3 and DS *et al*, manuscript in preparation) indicate that Ama102 cells possess an RNA polymerase II which retains about 30% of its activity in 0.1 $\mu\text{g ml}^{-1}$ α amanitin, a concentration sufficient to inactivate completely the Ama⁺ polymerase II. If α amanitin has a similar inhibitory action *in vivo* and *in vitro*, the normal growth of Ama102 cells in α amanitin coupled with the partial resistance of its RNA polymerase II activity to α amanitin *in vitro* seems paradoxical. Does the mutant Ama102 cell grow normally in α amanitin with only 30% of its normal RNA polymerase II active, or can the RNA polymerase II activity increase when the mutant is grown in α amanitin? The experiments reported here indicate that Ama102 cells can compensate for the partial inactivation of RNA polymerase II by α amanitin by increasing the RNA polymerase II activity.

The activity profile of the RNA polymerases obtained by DEAE-Sephadex chromatography of extracts of Ama102 cells grown for many generations in the absence of α amanitin

(Fig 1a) shows that polymerase II is partially resistant to 0.1 $\mu\text{g ml}^{-1}$ α amanitin. When these cells were grown for 4 d' in the presence of α amanitin (3 $\mu\text{g ml}^{-1}$) the polymerase II activity was completely resistant, representing a threefold increase in resistant activity (Fig 1b). There seemed to be no corresponding increase in polymerase I activity. The Ama102 cells responded to the exposure to α amanitin by modulating the activity of resistant polymerase II. This increased level of α amanitin-resistant RNA polymerase II activity was maintained even after 30 d of continuous growth in α amanitin (data not shown).

The α amanitin sensitivity of RNA polymerase I and II purified by DEAE-Sephadex chromatography from Ama⁺ and Ama102 cells grown with and without α amanitin was characterised further (Fig 2). RNA polymerase I from both wild type and mutant cells was not inhibited by up to 50 $\mu\text{g ml}^{-1}$ α amanitin, while the Ama⁺ RNA polymerase II was inhibited completely by 0.1 $\mu\text{g ml}^{-1}$. About 30% of the RNA polymerase II from Ama102 cells grown in the absence of α amanitin and all of it from Ama102 cells grown in 3 $\mu\text{g ml}^{-1}$ α amanitin was resistant to 0.1 $\mu\text{g ml}^{-1}$. This α amanitin-resistant RNA polymerase II activity was only inhibited by much larger concentrations of α amanitin (50% inhibition with 5 $\mu\text{g ml}^{-1}$). This α amanitin-resistant activity is distinguished from RNA

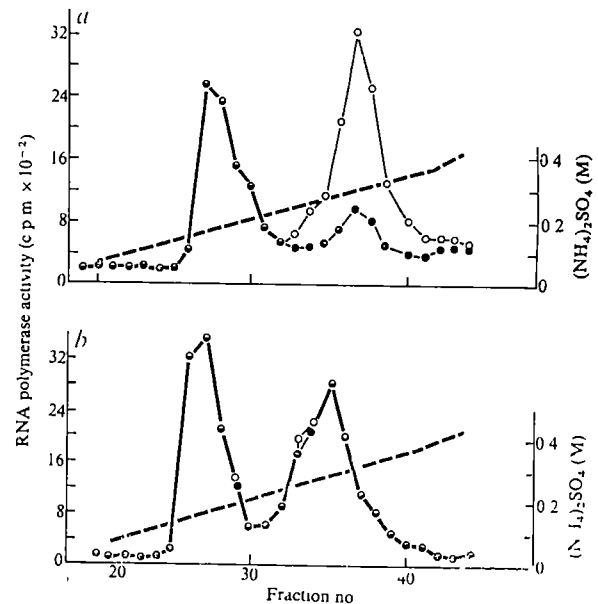


Fig 1 DEAE-Sephadex chromatography of RNA polymerase activities in α amanitin-resistant rat myoblast Ama102 grown in the absence and presence of α amanitin. Ama102 cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% foetal calf serum (Gibco) at 37°C under 100% humidity in 5% CO₂-95% air. 10⁸ cells, washed once with citrate saline, were resuspended in TGMED (50 mM Tris-HCl (pH 7.9 at 22°C), 25% (v/v) glycerol, 5 mM MgCl₂, 0.1 mM EDTA and 1 mM dithiothreitol) containing 10⁻⁴ M phenylmethylsulphonylfluoride. Ammonium sulphate (4 M, pH 7.9) was added to a final concentration of 0.3 M, and the suspension was sonicated, diluted with 2 volumes of TGMED and centrifuged (30 min, 4°C, 125,000g). The supernatant was diluted with TGMED to 0.03 M in ammonium sulphate and used for chromatography on DEAE-Sephadex A-25 (0.9 × 12 cm), eluting with 10 ml of TGMED, 0.03 M in ammonium sulphate, then a 40 ml linear gradient, 0.03-0.5 M ammonium sulphate in TGMED. RNA polymerase activity was determined in column fractions (1 ml) by addition of 40 μl of enzyme solution to 80 μl of assay mixture⁹ which contained 2 μCi ³H-UTP (18-22 Ci mmol⁻¹). The reactions, 20 min at 30°C, were terminated by adding 0.01 ml 5% (w/v) sodium dodecylsulphate containing 0.25 M sodium pyrophosphate. 100 μl of the reaction mixture was spotted on 24 cm Whatman DE-81 filter disks and the filters were washed and counted². a, Cells grown in the absence of α amanitin, b, cells grown for 96 h in the presence of α amanitin (3 $\mu\text{g ml}^{-1}$). RNA polymerase activity was assayed in the absence of (○) and presence of (●) α amanitin (0.1 $\mu\text{g ml}^{-1}$).

polymerase III both by its elution from DEAE-Sephadex before the small amounts of polymerase III detected (Fig 1) and by its inhibition by concentrations of α amanitin lower than those reported to inhibit polymerase III⁵

We have examined the time course of the increase in α amanitin-resistant RNA polymerase II activity in Ama102 cells (Fig 3) Cells grown in the absence of drug for many generations were put into α amanitin ($3 \mu\text{g ml}^{-1}$) and the total

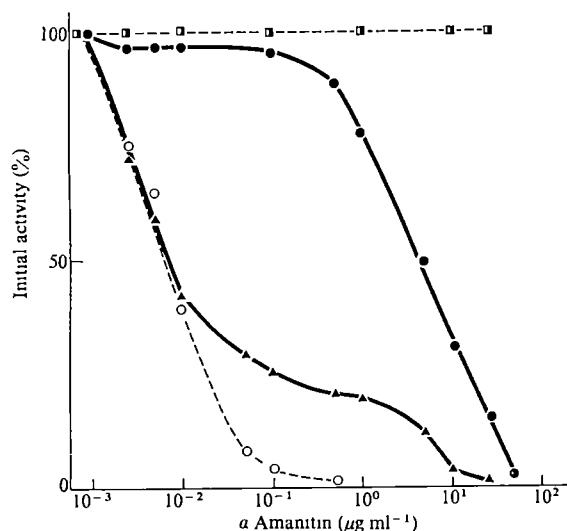


Fig 2 α Amanitin sensitivity of RNA polymerase activities from Ama102 and Ama⁺ cells purified on DEAE-Sephadex. Assay conditions were as described in the legend to Fig 1 except that each assay contained $5 \mu\text{Ci } ^3\text{H-UTP}$. \square , RNA polymerase I from Ama⁺, \blacksquare , RNA polymerase I from Ama102 grown for 96 h in α amanitin ($3 \mu\text{g ml}^{-1}$), \circ , RNA polymerase II from Ama⁺, \blacktriangle , RNA polymerase II from Ama102 cells grown in the absence of α amanitin, \bullet , RNA polymerase II from Ama102 cells grown for 96 h in α amanitin ($3 \mu\text{g ml}^{-1}$)

and α amanitin-resistant RNA polymerase activities in cell lysates were determined at various times under assay conditions where RNA polymerases I and III together accounted for no more than 17% of the total activity measured (D S *et al*, unpublished). Within the first 8 h there was a decline in the total specific activity (per mg total protein) of the RNA polymerase, probably resulting from the entry of α amanitin into the cells with consequent inhibition of RNA polymerase II activity. Within 16 h, however, the total RNA polymerase specific activity had increased to approximately its original value. This was entirely accounted for by an increase in α amanitin-resistant RNA polymerase activity. After 48 h of growth in α amanitin only α amanitin-resistant polymerase activity was detected in cell lysates. Removal of the α amanitin at 48 h led to a progressive decrease in the specific activity of α amanitin-resistant polymerase, the total RNA polymerase activity remaining constant. Fifty-six hours after removal of α amanitin, the levels of total and α amanitin-resistant RNA polymerase had returned to the prechallenge levels. The rapid change in the relative level of α amanitin-resistant RNA polymerase argues against the trivial possibility that these changes were due to selection of Ama^r and Ama^s subpopulations of cells within the Ama102 culture. Similar phenotypic regulation of RNA polymerase II activity has been observed in our laboratory in several other clones of independently isolated Ama^r myoblast mutants (M Crerar, S Andrews, and D S, unpublished).

These data indicate that RNA polymerase II activity in Ama102 is subject to regulation which is independent of that of the activity of RNA polymerase I. We suggest that RNA polymerase II synthesis in rat myoblasts is regulated autogenously as RNA polymerase synthesis seems to be in bacteria⁶. Based on experiments using *E. coli* *rif^r/if^s* merodiploids⁷ and *λif* infected *Escherichia coli*⁸ it has already been suggested

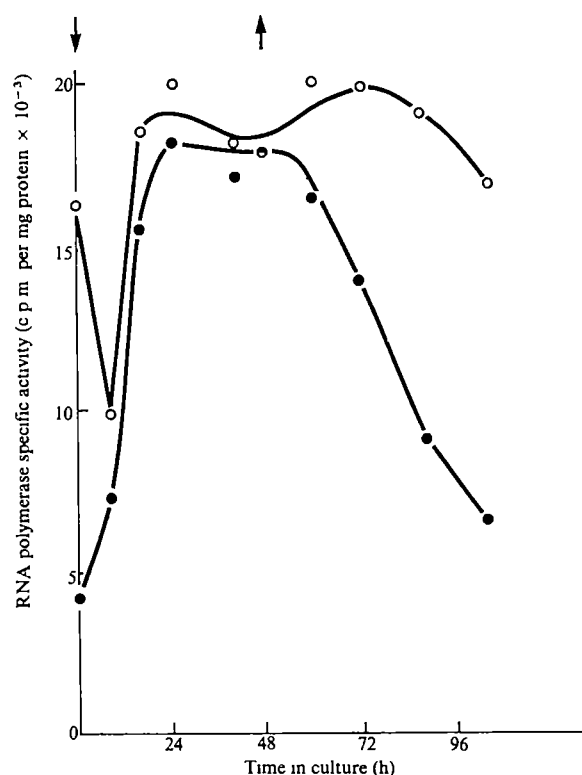


Fig 3 Changes with time of the RNA polymerase-specific activities in cell lysates of Ama102 cells grown in the presence and after removal of α amanitin ($3 \mu\text{g ml}^{-1}$). Ama102 cells, seeded in Falcon flasks (75 cm^2) at different densities to yield approximately 3×10^6 cells per flask when collected, were grown in the drug for various times up to 48 h. α Amanitin was removed from other flasks after 48 h, the cells were washed and fresh medium minus the drug was added. Ama102 cells were collected again at various times after removal of drug. The specific activities of RNA polymerase in the presence and absence of α amanitin ($0.1 \mu\text{g ml}^{-1}$) were determined in the cell lysates (3×10^6 cells suspended in 0.5 ml TGMED) prepared by four cycles of freezing and thawing (using liquid nitrogen and a 30°C water bath). The assay conditions were as described in Fig 1, except that each assay was made 0.4 M in ammonium sulphate to enhance the relative activity of RNA polymerase II compared with RNA polymerase I (D S *et al*, manuscript in preparation). Total protein was determined in these lysates by the method of Lowry *et al*¹⁰. The doubling time of Ama102 cells was 15 h in either the presence or absence of $3 \mu\text{g ml}^{-1}$ α amanitin. \circ , Total RNA polymerase activity, \bullet , α amanitin-resistant RNA polymerase activity. Downward arrow, addition of α amanitin, upward arrow, removal of α amanitin in the culture media.

that RNA polymerase may act as its own repressor. Our results suggest that this mode of gene regulation may extend to eukaryotes as well, although other mechanisms such as enzyme modification and end product feedback inhibition may regulate RNA polymerase II activity. A first requirement of this hypothesis of autogenous regulation will be the correlation of the increase in α amanitin-resistant polymerase II activity with an increase in *de novo* enzyme synthesis.

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A blocked structure at the 5' terminus of mRNA from cytoplasmic polyhedrosis virus

METHYLATION of a specific nucleotide occurs at the initial stage of transcription of the double-stranded RNA genome in cytoplasmic polyhedrosis virus (CPV) in the presence of the methyl-group donor, S-adenosyl methionine (SAM)¹. Methylation of mRNA has been reported recently not only for other viruses^{2,3} but also for a mouse L-cell⁴. Since methylation seems to be coupled with the initiation of mRNA synthesis in CPV, the structure of the initiation (5') terminus of mRNA transcribed from CPV by the virus-containing RNA polymerase is of particular interest.

In the absence of SAM, single-stranded RNA transcribed from CPV *in vitro* has the terminal structure ppA-G-Y-. All ten kinds of mRNA, which correspond to the ten genome segments, have the same terminal structure⁵. The mRNA has the same sequence as the 5'-terminal-modified strand of the double-stranded RNA genome in CPV⁷ as well as in reovirus⁸. When SAM is added to the *in vitro* reaction mixture of CPV transcription, RNA synthesis is greatly stimulated⁷. The RNA synthesised in the presence of SAM is the same size as the RNA transcribed in the absence of SAM, but the former is methylated in the ribose moiety (probably the 2'-position) of an adenylic acid residue in the 5'-terminal region¹. We report the structural analysis of the 5'-terminal part of the RNA synthesised in the presence of SAM, showing that the 5'-terminal adenylic acid residue is methylated in the ribose moiety and, further, that the 5'-terminal phosphates are blocked by 7-methylguanylic acid.

Isolation of CPV and its RNA^{9,10}, construction of the system for *in vitro* RNA synthesis in the presence of SAM¹, and analytical methods for the oligonucleotides^{6,7}, have been published previously.

The mRNA synthesised from CPV in the absence of SAM carries α,β -diphosphates at the 5'-terminal adenosine⁶. Also in the presence of SAM, β -³²P-labelled ATP is incorporated into the newly synthesised mRNA. CPV mRNA doubly labelled with β -³²P-ATP and ³H-methyl SAM was prepared. When this was digested by various ribonucleases or alkali, both the radioactivities of ³²P and ³H were always found in one component in the DEAE-cellulose-urea column chromatography (details will be published elsewhere). This means that methylation occurs only at the 5'-terminus. These experiments suggest that the 5'-terminal sequence is ppAm-G-Y-. The terminal nucleotide (ppAm) obtained by *Penicillium* nuclease^{7,11} (see Fig. 3) was not, however, changed by the phosphomonoesterase treatment (Fig. 1a). Therefore, phosphate groups at the 5'-terminal nucleotide must be blocked by some unknown material, X, as XppAm.

This 5'-terminal material (XppAm) was then treated with venom phosphodiesterase. The products were separated by paper electrophoresis. As shown in Fig. 1b five components were detected by radioactivity, one of which (III) was non-degraded original material. Component II was identified as pAm (2'-O-methyladenosine-5'-phosphate). This contains a ³H-methyl group and moves with standard pAm, synthesised chemically¹², on paper chromatography. When this was digested with phosphomonoesterase, ³H-radioactivity was detected in 2'-O-methyladenosine. Component V is inorganic phosphate

carrying radioactivity (³²P). Component I at the origin (charge zero) was considered to be phosphorylated material X (Xp) because phosphomonoesterase treatment for this gave a material X, which moves to the cathode faster than any normal nucleoside at pH 3.5, indicating that it has a positive charge. From other experiments (details will be published elsewhere) the material X is labile in alkali, and contains a *cis*-diol. As candidates for X, SAM and 7-methylguanosine (m⁷G) were chromatographed and electrophoresed with ³H-methyl-X. In each case the radioactivity of ³H-X was simply superimposed on the ultraviolet-absorbing spot of 7-methylguanosine (examples are shown in Fig. 2). Therefore, X must be 7-methylguanosine.

In component I, the plus charge of X seems to be cancelled by the minus charge of a phosphate group at pH 3.5. As this phosphate did not carry radioactivity, this is not the β -³²P of the terminal 2'-O-methyladenylic acid. When α -³²P-labelled

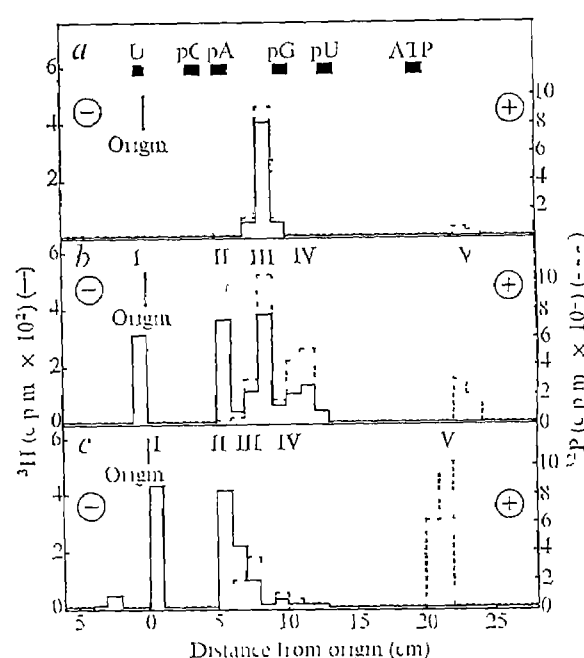


Fig. 1 Paper electrophoresis of the terminal oligonucleotide and its phosphodiesterase digest. *a*, Oligonucleotides obtained by *Penicillium* nuclease and phosphomonoesterase. CPV mRNA solution (100 μ l, ³H 60,000 c.p.m., ³²P 60,000 c.p.m.) was mixed with 5 μ l 0.1 M sodium acetate buffer (pH 6.0) and 20 μ l *Penicillium* nuclease solution, and incubated at 37°C for 90 min. The digest was spotted on Whatman 3MM paper and electrophoresed in pH 3.5 buffer (5% acetic acid, pH adjusted with morpholine), containing 5 mM EDTA, at 40 V cm^{-1} for 50 min in cooled hexane. After drying in a gentle air stream, the paper was cut into pieces (1 cm wide) and the radioactivity in each piece counted in 5 ml PPO- and POPOP-containing toluene. When the sample (20 μ l) was further mixed with 1 μ l phosphomonoesterase solution after adjusting pH to 8.0 with 1.0 M Tris-HCl buffer (pH 8.0) and incubated at 37°C for 30 min, a similar electrophoretogram was obtained. This means that the phosphate groups in the sample oligonucleotide are protected from phosphomonoesterase. *b*, Phosphodiesterase digest of *a*. The radioactive region in an electrophoretogram shown in *a* was extracted with 150 μ l distilled water. This sample (75 μ l) was mixed with 10 μ l 0.5 M phosphate buffer (pH 7.2), 1 μ l 1 M MgCl_2 , 2 μ l venom phosphodiesterase (Worthington, 7.5 mg ml^{-1}), and distilled water to total 200 μ l. The mixture was incubated at 37°C for 7 min. Paper electrophoresis as in *a*. *c*, Stronger digestion of *a* with phosphodiesterase. The same sample as the starting material in *b* was mixed with 20 μ l venom phosphodiesterase (15 times the concentration of *b*) and buffer containing MgCl_2 (same concentration as *b*) in 130 μ l total. The mixture was incubated at 37°C for 30 min. Paper electrophoresis was carried out at 36.4 V cm^{-1} for 50 min, otherwise as in *a*.

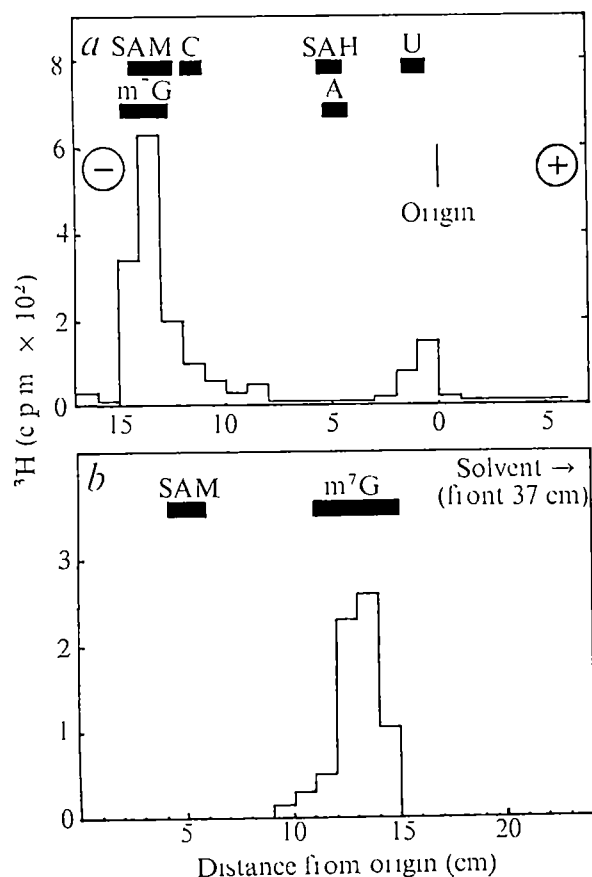


Fig 2 Identification of 7-methylguanosine linked to the terminal part of CPV mRNA. Fraction I in the phosphodiesterase digest of the terminal part of CPV mRNA, obtained from the *Penicillium* nuclease digest of the labelled mRNA (b), was eluted with distilled water, and digested with phosphomonoesterase under the same conditions as in Fig 1 a. The digest was spotted on Whatman 3MM paper and electrophoresed at 50 V cm^{-1} for 40 min in the same buffer as used in Fig 1 b. The digest was spotted on Whatman 3MM paper and developed with *n*-butanol-acetic acid- H_2O (5:1:4, V/V/V). As reference materials, SAM, S-adenosylhomocysteine (SAH), 7-methylguanosine, adenosine, cytidine, uridine, and the mixture of 5'-nucleotides (latter not shown) were run with the sample. Counting as in Fig 1.

guanosine-5'-triphosphate was added as a substrate, ^{32}P was incorporated into this component I. Therefore, component I is 7-methylguanosine-5'-phosphate (pm^7G). Component IV was considered to be the material X combined with two phosphate groups. By phosphomonoesterase treatment, ^{32}P was released as inorganic phosphate, together with ^3H -methyl-X. Thus component IV must be component I (Xp) further phosphorylated with ^{32}P phosphate which was incorporated from β - ^{32}P -ATP, that is, IV must be Xp^{32}P . Two phosphate groups would yield a minus charge for IV. Components III and IV in Fig 1b seem to remain because of incomplete hydrolysis by phosphodiesterase, as these components diminish when stronger conditions for hydrolysis are applied (Fig 1c). In the latter case there are three major components: I, pm^7G - ^3H -methyl, II, pAm - ^3H -methyl, and V, p^{32}P -phosphate.

Since the phosphate groups are blocked and protected from the phosphomonoesterase attack, 7-methylguanosine-5'-phosphate must be linked to the β -phosphate of ppAm through a pyrophosphate linkage. In fact pyrophosphatase treatment on the starting material yields 7-methylguanosine-5'-phosphate. Therefore, the starting 5'-terminal material has the structure $\text{m}^7\text{Gp}^5\text{pp}^5\text{Am}$ as shown in Fig 3. The number of phosphates between X and Am must be three, as the plus charge in m^7G was cancelled with one negative charge from phosphates

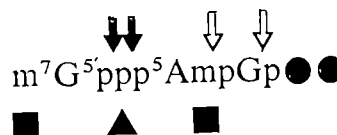


Fig 3 A proposed structure of the 5' terminal part of CPV mRNA and the enzyme-attacking sites. Radioactive components (\blacksquare , ^3H -methyl, \blacktriangle , ^{32}P). White arrow, attacking of *Penicillium* nuclease, black arrow, attacking of venom phosphodiesterase (after *Penicillium* nuclease digestion).

This consideration explains clearly the behaviour of the 5'-terminal material in DEAE-cellulose-urea column chromatography and in paper electrophoresis. Recently a similar blocked structure was reported for the 5'-terminus of low molecular weight nuclear RNAs from Novikoff hepatoma ascites cells¹³.

According to this analysis of the terminal nucleotide of CPV mRNA synthesised in the presence of SAM, the methylation occurs exactly at the initiation point (5'-terminus) of mRNA, as one of the genome duplex strands (ref 7 and K M and Y F, unpublished). In addition to the methylation of the ribose moiety of the terminal adenosine, 7-methylguanylic acid combines with the 5'-terminus of RNA by the 5'-5' pyrophosphate linkage. These modifications at the 5'-terminus of mRNA could play an important role in the initiation of mRNA synthesis and/or the function of mRNA. The blocked structure as well as the methylated 5'-terminal nucleotide of mRNA found here would be resistant to exonucleolytic attack, lengthening the life of mRNA. Alternatively, this modified structure may be necessary for completion of transcription, transportation and/or translation by forming a specific tertiary structure. Although the biological meaning of this modification of mRNA structure is not clear now, a similar modification has also been found in mRNA of vaccinia virus containing DNA as its genome¹⁴.

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Different effector cell types in antibody-dependent cell-mediated cytotoxicity

ANTIBODY-DEPENDENT cell-mediated cytotoxicity has been demonstrated *in vitro* using a number of continuously cultivated cell lines^{1,2} and nucleated erythrocytes³ as targets. Although the biological role of this cytotoxicity is not yet clear, it represents a potentially efficient cytotoxic mechanism and considerable interest has been shown in the characterisation of the effector cells.

The use of antibody-coated chicken erythrocytes (E-Ab) as targets has technical advantages and they have been widely used in assaying antibody-dependent effector cells. The report that effector cells in mouse spleen are monocyte-like⁴⁻⁶ was not easily reconciled with observations in our laboratory and elsewhere using cell line cells as targets. These authors assumed that the effector cells in mouse spleen active against E-Ab are the same as the effectors active against antibody coated cell line targets. It has been suggested that polymorphonuclear cells may participate in the reaction but even when these cells have been eliminated the possibility remains that more than one effector is involved. We have, therefore, made a direct comparison of the cells killing antibody-coated erythrocytes with those killing cell line cells coated with homologous antibody.

The results show that a population of effector cells highly active against antibody-coated erythrocytes has little or no activity against antibody-coated cell line cells.

The cytotoxicity assay towards antibody-coated chicken erythrocytes (E-Ab) was performed essentially as described by others^{3,6}. In addition two cell lines have been studied, the mouse mastocytoma P815 (M) and the human FL cell line (F)⁷. Each target was tested in the presence and absence of the appropriate antiserum at a dilution of 1:1,000, rabbit anti-E, rat anti-M and rat anti-human

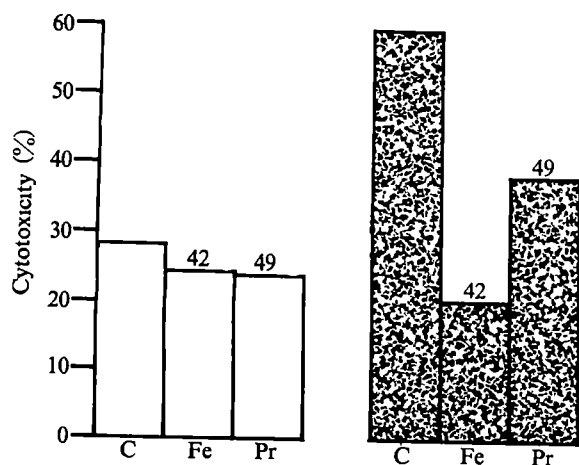


Fig. 1 Treatment of spleen cells with carbonyl iron. Protein coating was carried out by suspending 200 mg sonicated carbonyl iron in 10 ml of medium containing 10% foetal calf serum and incubating on a roller at 37°C for 60 min. Uncoated particles were treated in serum free medium. 100 mg of iron was added to 5×10^7 cells in 1 ml in a tube and incubated at 37°C for 30 min. The unadhered cells were removed with the aid of a magnet and the iron particles resuspended in medium. These washings were recovered in the same way, pooled with the first cell suspension and adjusted to 5×10^6 ml⁻¹. C, Untreated control, Fe, uncoated iron particles, Pr, protein-coated iron particles. The percentage of cells recovered relative to the controls is shown at the top of the histograms. Clear blocks are M-Ab cytotoxicity, hatched blocks are E-Ab cytotoxicity.

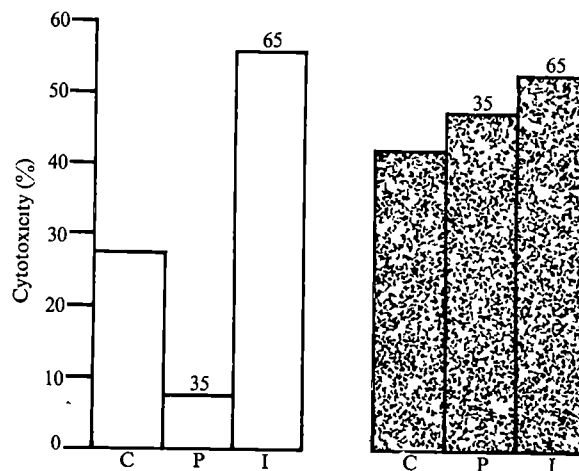


Fig. 2 Fractionation on Ficoll-Trisil®. 5 ml of cell suspension was layered over 10 ml of Ficoll-Trisil and centrifuged for 10 minutes at 500g. Cells from the interface (I) and the pellet (P) were washed and suspended at 5×10^6 ml⁻¹. The percentage of total recovered cells in the pellet and interface is indicated at the top of the histograms. Total cell recovery was 80%. Clear blocks are M-Ab cytotoxicity, hatched block are E-Ab cytotoxicity.

erythrocyte. Except where stated the concentration of target cells was 10^7 ml⁻¹ and of effectors 5×10^6 ml⁻¹, the percentage ⁵¹Cr release was determined after mixing equal volumes of effectors and target cell suspensions and incubating for 4 h at 37°C. The percentage of cytotoxicity was calculated as (test-control)/(maximum-control) using as control the isotope release in the absence of antibody and the values for maximum release obtained by lysis of E in water (approximately 65%) and twice freezing and thawing the cell lines in hypotonic solution (approximately 80%). The data have been analysed by a multi-variable analysis of variance using logarithmic transformation of percentage ⁵¹Cr release. All the values presented as cytotoxicity represent killing significant at the 1% level.

In all the experiments the same preparation of effector cells was tested on the different targets under identical conditions. The results with the two cell lines were similar in each case except that the mastocytoma was more susceptible to killing.

In our hands mouse spleen cells show high activity against E-Ab but only low activity against antibody coated cell line cells. For example, BALB/c spleen cells tested at a ratio of 50:1 for 4 h on E-Ab, M-Ab and F-Ab gave 47%, 3% and no detectable cytotoxicity respectively, whereas under the same conditions Agus rat spleen cells gave 48%, 27% and 10% respectively. This conclusion is supported by other work (for example ref. 8) using antibody coated cell line cells and mouse spleen effectors where high ratios and a long incubation period (100:1, 16 h) were necessary for cytotoxicity. Other experiments (C. J. S., to be published) established that the difference between the two species was not the result of the source of antibody as similar results were obtained with mouse antibody. Because of this relative deficiency in mouse spleen we have used Agus rat spleen effectors in these experiments.

Treatment of spleen cells with carbonyl iron considerably reduces the activity towards E-Ab while only slightly reducing and sometimes enhancing the activity towards M-Ab. The cell recoveries suggested that this technique

was removing more than just the phagocytic cells. In an attempt to differentiate between phagocytosis and adherence the iron particles were first coated with protein. Examination under the microscope indicated that the coated particles were readily phagocytosed. Figure 1 shows an experiment in which uncoated and protein-coated iron particles were used. Uncoated iron shows a differential removal of activity towards E-Ab whereas protein-coated iron shows very little differential removal of activity towards E-Ab. The reason for this is not entirely clear, protein coated particles consistently remove fewer cells than uncoated particles, although coated particles seem to be readily phagocytosed. This suggests that these effector cells are being removed by adherence rather than phagocytosis. In an experiment using BALB/c spleen effectors, activity against E-Ab was decreased from 47% to 7% by uncoated carbonyl iron, while the activity towards M-Ab remained unchanged (35% to 33%). Thus, in both species, the activity towards E-Ab is more adherent than the activity towards M-Ab.

Figure 2 shows the distribution of activity after centrifugation on Ficoll-Triosil interface. Under these condi-

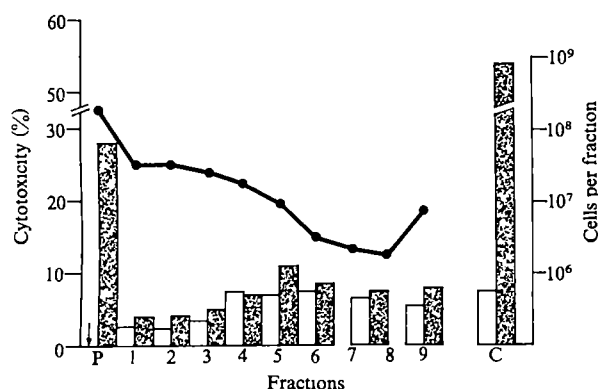


Fig 3 Fractionation on BSA gradient (modified from 10). 3.6×10^8 spleen cells were suspended in 1 ml of 35% BSA and placed in the bottom of a 10 ml centrifuge tube. A linear gradient from 35% to 20% BSA was formed over the cells. The tube was centrifuged in a swing out head (MSE Superspeed 65) at 20,000g for 30 min at 20°C. Approximately 1 ml fractions were collected from the bottom after puncturing the tube. The pellet was recovered with a Pasteur pipette. Cells were washed twice in serum free medium before counting and resuspending in medium at $5 \times 10^6 \text{ ml}^{-1}$. Cytotoxicity was determined after 5 h incubation. Cytotoxicity towards E-Ab is shown hatched, cytotoxicity towards F-Ab as clear blocks. Total cell recovery was 65% and the distribution is shown (●). Fractions 7 and 8 were pooled for the cytotoxicity assay. Unfractionated spleen control (C). The pellet (P) showed no activity against either cell line (arrow) but strong activity against E-Ab.

tions the cells recovered from the pellet are active against E-Ab with relatively little activity against M-Ab. The cells at the interface retain both activities.

In an attempt to make a better preparation of the lower density cells centrifugation was carried out at 1,000g. The cells recovered from the interface were then further purified by treatment with carbonyl iron. These cells gave 26.5% cytotoxicity with M-Ab and 75% with E-Ab.

These results indicate that the high density cells (pellet) active against E-Ab have little activity against M-Ab whereas the lower density cells with activity against M-Ab are less active against E-Ab. In each case it is not clear whether the residual activity against the other target cell is due to incomplete separation of the two types of effector.

In a further effort to improve the separation of the two types of effector cells we have investigated isopycnic centrifugation on different ranges of continuous bovine

serum albumin (BSA) gradients. Using a gradient from 35–20% BSA the cells recovered from the pellet show strong activity towards E-Ab with no detectable activity towards M-Ab or F-Ab. On the other hand, there is a population of cells with a peak at the middle of the gradient which is active towards all targets. Strong background killing of M in the absence of antibody was, however, also found in these fractions. The reasons for this are unclear (it may be caused by immune complexes in the BSA) but it made the calculations of cytotoxicity unreasonable and so only the data obtained with F-Ab are presented (Fig 3).

As the two cell lines we have used are quite different in origin and susceptibility to killing, it seems reasonable that other susceptible cell lines will be killed by the same population of effector cells. But the data obtained with E-Ab, at least with rodent spleen effectors, must be interpreted with caution and cannot necessarily be generalised to cell line targets.

Greenberg *et al* implied that treatment of mouse spleen with iron removed only cells with a phagocytic function, leaving non-phagocytic but adherent effector cells ("null cells"). It stems from our data and those of Dennert and Lennox¹¹ that the adherent properties of these cells can render them susceptible to almost total removal by iron, along with actively phagocytic cells. This effect may be responsible for the disagreement in the conclusions of various authors using E-Ab target cells^{6,11,12}.

In conclusion, rodent spleen contains two populations of antibody-dependent cytotoxic cells. One is a low density, non-adherent cell with activity towards cell line targets and chick erythrocytes. Mouse spleen is a relatively poor source of these cells compared with rat spleen. The second type of cell is a higher density, adherent cell. This is clearly the cell characterised by Greenberg *et al* as "null cells". These cells are present to a similar extent in both rat and mouse spleen, they are cytotoxic towards chick erythrocytes but not cell line targets.

We have avoided the suggested terminology (K-cells)¹³ because it is not clear whether it should be used to include both types of effector cell. We wish to thank Drs A C Allison and E M Lance for criticising the manuscript.

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Age limitation of perceptual span

DIFFERENCES in speed of perception and performance between younger and older human adults sometimes increase proportionately with task difficulty, but sometimes remain constant. Gregory¹ has argued that the added time is adaptive and has shown how a constant component, such as age-associated

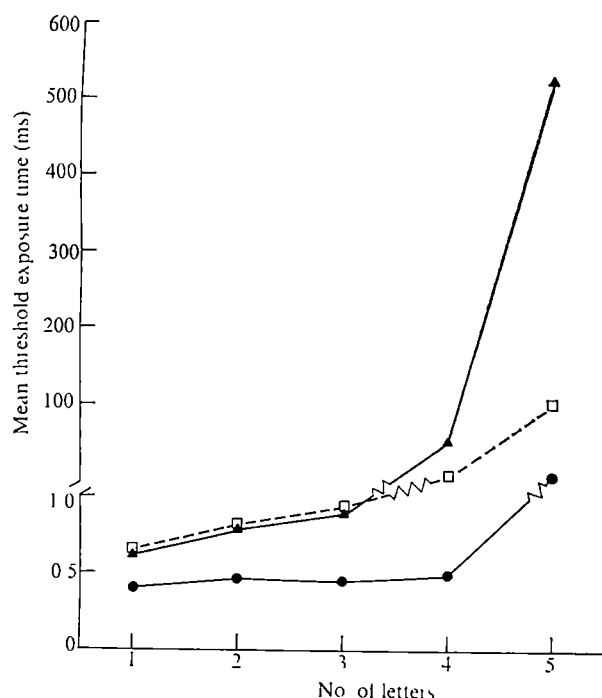


Fig. 1 Absolute thresholds of time exposure for correct identification of varying numbers of letters by different age groups ●, 20-30 age group, □, 40-50 age group, ▲, 60-70 age group

rise in 'noise' level, could result in a linear increase of decision time. Welford² suggests that the constant age effect occurs when signals are brief, whereas when perception is not limited, proportionate increases are manifested. We report here an example of a constant difference between age groups in absolute time thresholds followed by an abrupt change in a situation where signals are brief. The implication seems to be that two different deficiencies are operating, both of which could perhaps ultimately be attributed to 'noise'. Participants in the experiment were required to identify varying numbers of letters and the sudden increase in required time indicates a reduction in the visual perceptual span of older people.

A modified staircase method³ was used to determine the absolute time thresholds for correct reporting of 1, 2, 3, 4 and 5 upper case consonants. In estimating thresholds for more than 1 letter, correct order of report was required. Black stimuli (Letraset 719, 60-point spaced 10 mm centre to centre) were printed in the middle of a white card and displayed binocularly in a 3-channel tachistoscope with a dark pre- and post-exposure field. After every successful or unsuccessful trial the stimulus was replaced by a member of a set of 20 at each level of difficulty. Five male and five female volunteers in each of the age ranges 20-30, 40-50 and 60-70 were tested. To equate for age differences⁴, subjects in the young, middle and old age groups were dark adapted for 5, 7 and 10 min respectively, the experimental room having very low illumination (one 25-W red light bulb). Calculations are based on the mean of two estimates of threshold for each subject, order of presentation being 2, 3, 1, 4, 5, 3, 2, 4, 1 and 5 letters.

Results are given in Fig. 1 and show a dramatic increase in the exposure time required by the oldest group to identify 5 letters. Among that group there was only one exception to the very large time increment. Two members of the middle age group demonstrated a comparable increase, and there was one case in the oldest group with a similar increase at 4 letters. The youngest group had significantly lower thresholds than the two older groups for 1, 2 and 3 letters as well as a lower threshold than the oldest for 5 letters. The difference

between the middle and oldest group was only significant for 5 letters.

When stimuli were below threshold all age groups made most of their errors in the middle letters of a series. It is therefore unlikely that the limited perceptual span of the elderly is the result of poorer peripheral vision. For the same reason, output interference is contraindicated as the primary explanation, since such interference should lead to more frequent errors in the report of the last letter of a series. Nevertheless, a sensory memory storage deficit could be a major component in the reduction of perceptual span with age. This possibility is not excluded by the equivocal results of Abel's study⁵ using Sperling's⁶ partial report method, where exposure times of one half second were used. Many older participants in the present experiment made statements implying that they had seen a letter, but that it had disappeared before it could be identified. Similar comments have been reported from younger subjects in previous investigations⁷ and were made by members of younger age groups in a study we ran on time thresholds for more than five letters. The sudden increase in time we found with 5 letters among older people occurred at 6 or 7 letters in the young. Here, too, sensory storage is likely to be a major factor in limiting perceptual spans. Indeed, it would not be wrong to describe the perceptual span as a sensory memory span, since readout follows the disappearance of the stimulus and therefore must be formed from a lingering image.

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Errata

In the letter "Inhibition of adenylyl cyclase by an exotoxin of *Bacillus thuringiensis*" by D. G. Grahame-Smith, P. Isaac, D. J. Heal and R. P. M. Bond (*Nature*, **253**, 58, 1975) Figs 1 and 2 were transposed. The legends are correct as they stand.

In the letter "Sustained oscillations of acetylcholine during nerve stimulation" by Y. Dunant, P. Jirouneck, M. Israel, B. Lesbats and R. Manaranche (*Nature*, **252**, 485, 1974) the label on the abscissa of Fig. 1a should read 'Time (s)' and not as printed.

In the article "New observations of the angular diameter-redshift relation for radio sources" by A. Hewish, A. C. S. Redhead and P. J. Duffett-Smith (*Nature*, **252**, 657, 1974) there is a misprint on page 659. In line 18, 0.17" \pm 0.3" should read 0.17" \pm 0.03".

In the article "Two types of resistance to polyene antibiotics in *Candida albicans*" by C. C. Hsu-Chen and D. S. Feingold, (*Nature*, **251**, 656, 1974), the following corrections should be made to the legend of Table 2. In line 2, for CH₃COOH read MeOH, the expression in lines 4 and 5 should read [(c.p.m. of given phospholipid)/(total c.p.m. in phospholipid fraction)] \times 100, and not as printed.

matters arising

Simplification of palindromic telomere theory

CAVALIER-SMITH's motive in devising his palindromic model for telomeres¹ was to surmount the obstacle of the replication of the 3' end of a linear molecule of DNA (Fig 1). His palindromic end with related hairpin bend, however, makes the 3' end unnecessary and therefore, by the usual rules of natural selection, improvable.

This can be achieved by a slight modification of his original model (Fig 1). This has the attraction of not only being simpler, five steps replacing seven, but also of explaining the special property of telomeres which distinguishes them from any other end of a linear DNA molecule, namely a chromosome break their stability.

The essence of my model is that the self-paired telomeric palindrome is the normal condition, in G₁, G₂ and M. Then the phosphate backbone of the double helix is continuous through the telomere. Similarly, DNA replication

will proceed continuously through the telomere thus making the terminal RNA primer redundant.

This self-paired state is temporarily lost as the result of S and restored via denaturation. The endonuclease that nicks the end of the palindrome and allows the unfolding and refolding in the new configuration must be telomere specific, making it probable that all telomeres of one genome carry the same palindrome. Without this manoeuvre sister chromatids would remain attached end-to-end leading to non-disjunction at anaphase.

A philosophical attraction of my modification of the model is that the sister molecules (sister chromatids) participate equally in the process, in contrast with the original model in which one complete molecule is produced at once and the sister molecule is incomplete and has to go through a complex manoeuvre to correct itself. My model also requires one S phase, at the start. The original model starts and ends with DNA synthesis.

Finally, my model may be open to experimental confirmation. If the telomeric palindrome is long enough, the asymmetry between sister telomeres, one being old and one wholly new, might be detectable in autoradiographs of the first metaphase after labelling in S with ³H-thymidine. Wilson and Thomas report palindromes up to 12,000 nucleotides long². As there are only two telomeres per chromosome the possibility of recognising telomeric palindromes in DNA extracts would be like searching for the proverbial needle (or should we say hairpin?) in a haystack.

This work was supported by the Cancer Research Campaign and the Medical Research Council.

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¹ Cavalier-Smith, T, *Nature*, **250**, 467-470 (1974)

² Wilson, D A, and Thomas, C A, *J molec Biol*, **84**, 115-144 (1974)

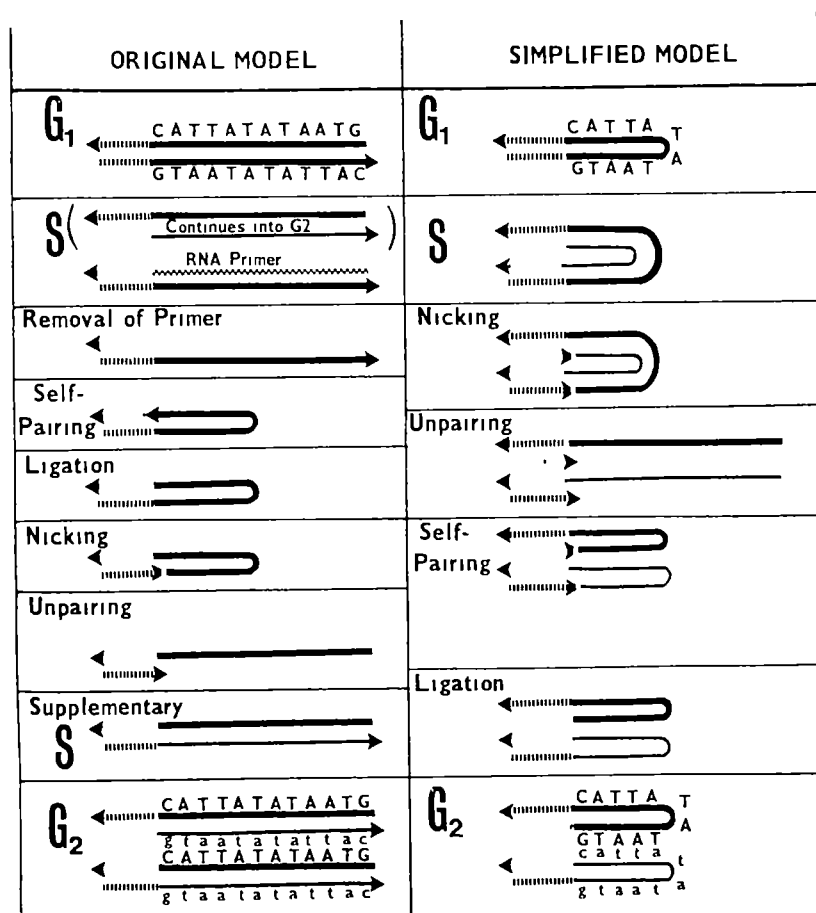


Fig 1 Two models for the replication of telomeres. —, Old DNA, —, new DNA, ---, RNA. The arrowheads indicate the 3' ends of the phosphate backbone. Capital letters nucleotides of old DNA. Small letters, new DNA. Entire line, the palindrome. Dotted line, the rest of the DNA molecule (of indefinite length).

- DR CAVALIER-SMITH REPLIES—I like Bate-man's modification of my model. It implies that a non-replicating eukaryote chromosome is a single, circular self-complementary polynucleotide chain, there are several ways this might be tested. In fact recent evidence indicates that vaccinia virus DNA may have this structure¹. Some other viruses do not, however, and an enzyme system has been isolated² from phage T7 which can cross-link the termini of some of them (T7, T3, T1 and λ) in the ways postulated in my original paper³.

This implies that even these phages have terminal palindromes, they may therefore be able to replicate according to my original model as well as (or instead of) Watson's concatomeric one. Another model involving a terminal palindrome has been independently proposed⁴ for the replication of linear mitochondrial DNA in *Tetrahymena*. But this postulates a palindrome only at one end and therefore requires a circular intermediate, and so seems implausible for eukaryote nuclear DNA.

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Hepatocyte sialoglycoprotein

EVANS¹ reported that the plasma membrane enzyme nucleotide pyrophosphatase is located on the external face of the hepatocyte surface. Based on this result, Evans argues that proposals which suggest cell surface glycosyl transferases function in cell adhesion^{2,3} and in selective binding of asialo-glycoproteins⁴ are less tenable.

As nucleotide pyrophosphatase on the outer membrane surface rapidly cleaves nucleotide sugars and Roseman² has placed an important role for these glycosyl transferase substrates in his theory of cell adhesion, Evans says the proposed mechanism would not be possible. I wish, however, to re-emphasise our results which implicate liver plasma membrane galactosyl transferase in the binding of asialo-glycoproteins⁴. We suggest that this result modifies Roseman's original proposal to the extent that cells remain attached to one another by complexes between glycosyl trans-

ferases and their oligosaccharide products. Thus, the liver recognition of non-reducing terminal galactosyl residues on asialo-glycoproteins does not require any involvement of nucleotide sugars, as the active transferase seems to be galactosyl transferase for which the asialo-protein is a product, and not a substrate.

Evans' result suggests to us that another possible importance for nucleotide pyrophosphatase being localised on the exterior of hepatocytes is that this enzyme may catalyse degradation of external RNA. Clearly a cell must exclude foreign messenger RNA from its cytoplasmic environment, as such a molecule might function in protein synthesis resulting in the eventual transformation of that cell. Such a potent effect of RNA has already been shown to be operative for RNA tumour viruses. Naked RNA itself can be infective⁵, and the addition of other macromolecules which may complex with the RNA and thus protect it from enzymatic hydrolysis enhances infectivity⁶. Results have shown⁷⁻⁹ that liver plasma membrane degrades RNA, and inhibition of membrane phosphodiesterase (nucleotide pyrophosphatase) with EDTA diminishes this activity^{7,9}. A second, well-established marker enzyme for the plasma membrane is 5'-nucleotidase and it also has been shown to be on the outside surface of both liver¹⁰ and leukocyte¹¹ membranes. The 5'-nucleotide products of nucleotide pyrophosphatase would be substrates for 5'-nucleotidase, and indeed liver plasma membrane completely hydrolyses RNA to nucleosides plus inorganic phosphate⁷.

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Matters arising

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DR EVANS REPLIES—Aronson is correct in pointing out that the binding of asialo-glycoproteins by a receptor, possibly a galactosyl transferase present in a liver plasma membrane fraction can occur without a requirement for sugar nucleotides. The involvement of sugar nucleotides in the transglycosylation reaction was postulated by Roseman to account for the de-adhesion process¹ and therefore their hydrolysis by the surface-located nucleotide pyrophosphatase would be a problem. Such proposals, however, must remain tentative until it is demonstrated more clearly that galactosyl and other sugar transferases are indigenous and functional components of the liver plasma membrane, and do not reflect contamination by Golgi membranes.

The plasma membrane fraction used by Aronson was shown to derive mainly from the liver sinusoidal surface² and to be contaminated by Golgi membranes as indicated by morphological markers and high activities of galactosyl and sialyl transferases^{3,4} that we have shown to be absent from the contiguous and bile canalicular face subfractions³. A fuller assessment of a possible role for cell surface galactosyl transferases and associated enzymes in the binding of serum components and in cell adhesion must await further analysis of the specific domains of the surface membrane and their interrelationships with Golgi membranes. Recently, the absence of cell surface galactosyl transferase in a number of cell lines was reported⁵, supporting the general conclusion that this enzyme does not play a role in cellular adhesion, cell recognition and contact inhibition.

Medical Research Council,
National Institute for Medical Research,
London NW7, UK

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- ² Wisher, M. H., and Evans, W. H., *Biochem. Soc. Trans.*, **2**, 407-408 (1974)
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reviews

Creative Malady. By George Pickering. Pp. 327+8 plates. (Unwin and Allan: London, 1974.) £5.25.

THE relationship between creativity and illness is one which stimulates the interest of most of us, particularly when an achievement is of the order of genius and seems to involve an impressive destructive action upon the subject's personal life. In this book Sir George Pickering, former Professor of Medicine at London University, attempts to elucidate this peculiar relationship by studying the lives of six famous people: Charles Darwin, Florence Nightingale, Mary Baker Eddy, Sigmund Freud, Marcel Proust and Elizabeth Barrett Browning.

The main hypothesis put forward by Sir George is that creative malady takes two quite distinct forms. In the first, exemplified by Darwin and Florence Nightingale, the illness serves a specific purpose: that of protecting the 'invalid' so that he or she can undertake the immense creative task undisturbed and unmolested. The second kind of creative illness can be observed in the lives of Freud, Proust and Mary Baker Eddy. In these cases productivity followed a period of "great turmoil and torment of mind" which were of sufficient degree to merit the term illness. Freud noted himself that he could not do creative work when he felt well and happy. The last subject in the book, Elizabeth Barrett Browning, unlike the others, showed no obvious connection in her life between creativity and illness.

Sir George gives most of his attention to the first of these types of illness as displayed by Darwin and Florence Nightingale. He has a rare gift for concise and vivid biography and these two lives make fascinating reading. Moreover, he is able to show clearly that the two subjects did indeed use their illnesses as a defensive barrier against forces which would have impinged on their work. (Both of them, for instance, became prostrated by palpitations and other symptoms in the face of intrusion by unwelcome friends, relations or public engagements.)

Creative Malady would, however, have been better if Sir George had focused throughout on that theme, for when he turns to other factors in creative illness he underestimates the complexity of the problem and is far less convincing. 'Creativity' and 'illness' are not simple and unambiguous con-

Genius and the mortal afflictions

"Creative genius arises, in a few rare people, out of an early despair of loving . . .

Therein lies the strength and ruthlessness of their drive.

Little wonder they are scarred or ill". Proust (pictured right) was only one of many so afflicted. Review by Peter Lomas



cepts and the two cannot be treated adequately as distinct entities. Take, for instance, Darwin's confessions about himself:

"I have said in one respect that my mind has changed during the last twenty or thirty years. Up to the age of thirty, or beyond it, poetry of many kinds, such as the words of Milton, Gray, Byron, Wordsworth, Coleridge, and Shelley, gave me great pleasure, and even as a schoolboy I took intense delight in Shakespeare, especially in the historical plays. I have also said that formerly pictures gave me considerable, and music very great, delight. But now for many years I cannot endure to read a line of poetry; I have tried lately to read Shakespeare, and found it so intolerably dull that it nauseated me. I have almost lost my taste for pictures or music.

This curious and lamentable loss of the higher aesthetic tastes is all the odder, as books on history, biographies, and travels (independently of any scientific facts which they may contain), and essays on all sorts of subjects interest me as much as ever they did. My mind seems to have become a kind of machine for grinding general laws out of large collections of facts, but why this should have caused the atrophy of that part of the brain alone, on which the higher tastes depend, I cannot conceive."

The impoverishment which Darwin describes could itself be considered either as a form of illness (in the sense of psychological disturbance) or as a state of mind liable to provoke manifest illness. How much impoverishment can a person take before becoming ill? To what extent was Darwin's neurosis caused by conflict between creative drive and the reluctance to pay the heavy price? And what is the origin of a drive so strong that it can, in many cases, wreak such personal havoc? Sir George does not really get to grips with questions of this kind and

that is because, in spite of his admiration for Freud, he places too little emphasis on early individual development. He comes closest to that in describing Proust's relationship with his mother.

Proust was 'in love' with his mother all his life and wrote *A la Recherche du Temps Perdu* only after her death, as if to fill a void. But I suspect that the real void began much earlier. Sir George quotes a moving passage from the great novel in which Proust describes his passionate dependence, as a child, on his mother's all-too-brief goodnight kiss. Sir George writes:

"Why then did Proust write a masterpiece while I have not? A difference of talent no doubt. Moreover, Proust was rich and never had to earn his living. While Proust was able to devote his whole talent to creating his book, I was leading an extremely busy life of practice, teaching, research and public affairs. But there is another great difference. While I was devoted to my mother, as he was to his, and while I had more need of her, since my father died when I was three, my *Recherche du Temps Perdu* would not have lingered on or indeed referred to any incident when my mother withheld the goodnight kiss. If she did so, I have no memory of it whatsoever."

That, I believe, is the crucial factor in an exceedingly complicated and little known phenomenon. Creative genius arises, in a few rare people, out of an early despair of loving. They create an alternative world as a substitute for the world they have lost. Therein lies the strength and ruthlessness of their drive. Little wonder they are scarred or ill.

Sir George may not have written a masterpiece. But he has given us a book which not only contributes to our understanding of creativity but also is a delight to read. □

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Storage diseases

Enzyme Therapy in Lysosomal Storage Diseases. (Proceedings of the Workshop on Cell Biological and Enzymological Aspects of the Therapy of Lysosomal Storage Diseases, Leiden.) Edited by J. M. Tager, G. J. M. Hooghwinkel and W. Th. Daems. Pp. xi+308. (North Holland: Amsterdam and Oxford; American Elsevier: New York, 1974.) Dfl65; \$25.00.

THE identification of the lysosomal enzymes which are lacking in various human, genetically-controlled storage diseases, transformed the approach to this subject. Now that there is a large measure of agreement on the classification and manifestations of these diseases hope is rising that something might be done to ameliorate the conditions of those who suffer from the consequent physical abnormalities, mental retardation, pain and death. Most of the storage defects can be manifested in individual cultured cells which have enzymes missing and in which substrates consequently accumulate. Several of the defects can be identified in cultures of amnion cells, so that prenatal diagnosis is now feasible, which allows the possibility of induced abortion of homozygous children in affected families.

In April 1974 a workshop on the possibilities of enzyme replacement therapy was held in Leiden, and the papers presented were collected for publication in this volume. Many are concerned with the purification and further characterisation of the relevant enzymes and isoenzymes. Others are concerned with *in vitro* complementation, when cultured cells with different defects are fused to form heterokaryons or, remarkably, simply pick up enzymes from each other to attain phenotypically normal characteristics. Since the defect is in the lysosomal system, it may seem relatively easy to restore the missing enzyme, but replacement therapy presents serious difficulties. Liposomes have been used to introduce hydrolases into cells, where they can break down lysosomal contents in model systems. These can protect enzymes from inactivation by antibodies in the circulating blood.

Various attempts have been made to cure deficiency diseases by allotransplantation. In Fabry's disease accumulation of trihexosyl ceramide is often associated with deterioration of kidney function and, according to Desnick, kidney transplantation results in a fall in plasma trihexoside levels and the alleviation of painful crises. Although the missing enzyme, α -galactosidase A, is present in the urine, it is not demonstrable in blood, and the substrate is thought to be filtered from the blood and degraded in the kidney.

Enzymes administered by parenteral injection do not reach the central nervous system, so that a major problem will be to alleviate the effects of storage diseases there. If, however, levels of substrates in the blood could be reduced, there could be some beneficial effect.

The book's usefulness is increased by its rapid publication, made possible because the photo-offset process was used. The standard of reproduction of line illustrations is excellent and even that of half-tone illustrations is acceptable. I suspect that this will soon become the standard way to publish meeting reports.

A. C. Allison

Introducing physics

Solid State Physics. (The Manchester Physics Series.) By H. E. Hall. Pp. xviii+351. (Wiley: London and New York, September 1974.) \$15.00; £7.50.

THIS fourth book in the Manchester Physics Series is intended for use in undergraduate courses. The author has divided the text into two parts. In the first five chapters he presents a self contained, but elementary, introduction to the basic concepts of solid state physics, which should also be suitable for chemistry students. The early sections include topics such as crystal geometry, lattice vibrations, transport in semiconductors, free electron theory and magnetism. The remainder of the book is aimed towards honours physicists and contains chapters on band theory, X-ray and neutron scattering, thermal conductivity and Fermi surfaces. As a result of this division the formal constructs of reciprocal space and Brillouin zones do not appear until chapter six. The first chapter also flouts convention since the author attempts to explain why solids take up their various lattice structures rather than simply listing the possibilities. Indeed, the book begins with a detailed account of the quantum mechanics of covalent and ionic binding and the author follows this general line of argument throughout. The last few chapters on superconductivity, magnetic ordering and disordered solids are concise, but nevertheless, instructive. The section on the Fermi surface is, however, somewhat spoiled by the unwarranted amount of space (for a book of this kind) which is given to Overhauser's work on possible instabilities of the electron gas.

Each chapter has a useful set of problems associated with it, and answers are provided. I can recommend this as a good, if somewhat unusual, introductory text book. For a full honours course, however, one would probably require a little more experimental and theoretical detail than is contained here.

R. Evans

From metal to insulator

Metal-Insulator Transitions. By N. F. Mott. Pp. xvi+278. (Taylor and Francis: London, September 1974.) £6.50.

In the early days of quantum mechanics a simple model of metals was introduced in which all valence electrons were assumed to be free, or 'nearly' free, when a weak interaction with the static lattice of the positive ions was allowed for. The model predicted that in a perfect crystal the electron states would lie in bands and that each band could accommodate two electrons in every atom. Thus, materials with odd numbers of electrons in each atom, or with occupied and empty bands that overlap, would be good conductors. An insulator could only be changed into a metal (at least at zero temperature) by making its bands overlap. That outstanding prediction survived the test of time. In a real solid, however, the electrons interact with one another and also respond to lattice vibrations and the conditions under which a metal-insulator transition may occur depend to a large degree on the nature of those interactions. The main theme of Professor Sir Nevill Mott's book is to describe the effect of interactions between electrons in inducing magnetic moments and metal-insulator transitions. He reviews the present state of knowledge in the field from both theoretical and experimental standpoints.

The book is divided into six chapters. In the first, the concept of metal-insulator transition is developed within the framework of the model of non-interacting electrons in crystalline and non-crystalline media. The second chapter introduces electron-phonon interaction and exciton formation into the discussion of metal-insulator transition. Chapter 3 deals with the theory of localised magnetic moments in metals, magnetic ordering in non-metallic oxides and metallic ferromagnets, and also outlines the physics of the Kondo effect and its relevance to the main theme of the book.

Chapter 4 covers the metal-insulator transition resulting from correlation. This forms the hard core of the book and includes perhaps its best written passages. It begins with Professor Mott's original argument that at zero temperature a small number of free carriers can always form bound pairs through the long-range Coulomb potential, thereby producing an excitonic insulator. As a consequence, the corresponding metal-insulator transition involves discontinuity in the number of carriers.

Metal-insulator transition can, however, also take place without any reference to the long-range forces. The

Hubbard model, in which only intra-atomic correlation is included, predicts a continuous change in carrier concentration in the region of metal-insulator transition. This theory and its numerous extensions are discussed in some detail. The chapter also describes transitions in highly correlated metals and Wigner and Verwey transitions. The last two chapters apply the ideas of the previous chapter to metal-insulator transitions in transition metal compounds (V_2O_5 , Ti_2O_3 , VO_2 , NiS , and so on) and in disordered systems (doped semiconductors, metal-ammonia solutions).

It must be remembered that although some of the most important contributions to the theory of metal-insulator transition were made many years ago (N. F. Mott, *Proc. Phys. Soc.*, **A62**, 416; 1949) the field is still very much a new one and, as Professor Mott himself points out, there is, among theoretical physicists, no consensus to speak of. Under such circumstances Professor Mott's attempts to give a unified view, embracing a wide variety of experimental and theoretical material, must be highly praised. The book will be a welcome source of up-to-date information (a list of over 500 references to scientific papers and books is also included) to postgraduate researchers in solid state physics. **M. Jaros**

Sequences

Handbook of Nucleic Acid Sequences. By B. G. Barrell and B. F. C. Clark. Pp. 104. (Joynson-Bruvvers: Oxford, May 1974.) £4.40 cased; £2.50 looseleaf.

THIS handbook is essentially a comprehensive catalogue of most of the known RNA and DNA sequences so far published and, of necessity, the bulk of the listings refer to the 50 or so tRNA species which have been sequenced. Another attractive target for sequencers are the 5S ribosomal RNAs and although little can yet be said about their function, the primary structures of nine different species of this molecule have been established. Several other low molecule weight (4.5S-6S) RNAs are included as well as the sequences of RNA transcripts from some DNA phages.

A second major section lists all the known sequences from coliphage group I and group III RNAs, and the third section shows the current state of the art of the DNA sequencer and includes a synopsis of DNA restriction enzyme cleavage sites. One would anticipate that the field covered in this section will expand rapidly and it is to be hoped that arrangements are in hand for the regular updating of this collection. **J. Hindley**

Developmental Biology books

1975



Development of the Avian Embryo

A Behavioural and Physiological Study
B. M. FREEMAN and M. A. VINCE

December 1974; 380 pages; 15 pages of plates and 110 line illustrations; hardback: 412 115204; £10.10

This book considers the behavioural and physiological development of the avian embryo. After describing the physical conditions needed for development both in incubators and in the wild, the first part goes on to describe the major changes which occur in embryonic physique, posture and activity in the course of incubation. The second part discusses the physiological aspects of development in detail. The book is intended primarily for the research student and the established research worker.

Differentiation and Growth of Cells in Vertebrate Tissues

Edited by G. GOLDSPIK

December 1974; 334 pages; 109 tone and 25 line illustrations; hardback: 412 113902; £10.30

This book brings to the fore a relatively neglected part of developmental biology as far as textbooks are concerned; namely the development of the cells in different tissues of the body. There are seven specialised chapters, and each chapter is written by an expert in that field and an all-round picture is given of the biochemical physiology as well as the morphological events associated with the development of that particular tissue. The volume will prove invaluable to postgraduates and advanced undergraduates of biology and medicine.

Cellular Interactions in Animal Development

ELIZABETH M. DEUCHAR

February 1975; 308 pages; 12 pages of plates and 126 line illustrations; hardback: 412 130106; £6.50

This book provides a broad and critical survey of the many types of interaction that have been shown to take place between cells during the processes of development in animals.

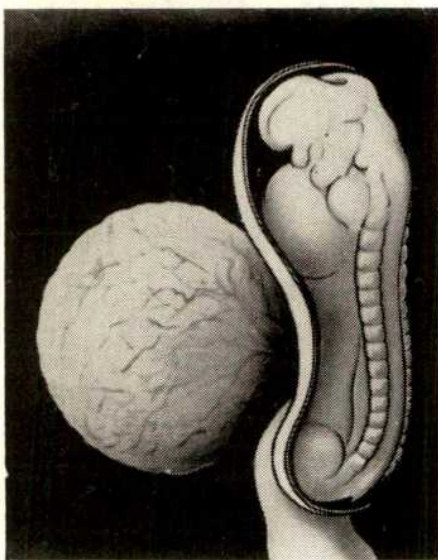
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• Film review

Looking at ourselves before birth

A 25 day old embryo, with its connecting stalk and yolk sac.

To describe the development of a human being from ovum to newborn baby in just over half an hour must inevitably mean an extensive compression of the available factual material. In *Ourselves Before Birth* (Macmillan Educational Films) the precis is unfortunately uneven. Ovulation, fertilisation, implantation and the early formation of tissue of a human embryo are described in the first third of the film and the rest of the time is devoted to the growth of the foetus and the development of its blood supply. That imbalance is, however, a major flaw in an otherwise admirable presentation.

As an exposition on foetal growth the film must be one of the best ever made, and the graphics are excellent. But as an educational aid for the study of human embryology in the first few days of life it is disappointing. The men behind the film, Professor W. J. Hamilton and Professor T. W. Glenister, of the Univer-

sity of London, have tried to cram far too much complex information in the first part of the commentary without explaining the technical terms introduced. If the members of an audience already know the meanings of these terms then they have no need to watch the first ten minutes or so of what is a rather superficial treatment; if they do not understand them then they will not be enlightened by this film.

So, for me, the film fell between two stools. As an undoubtedly excellent account of later foetal development it is burdened by technicalities relating to early embryology, which are not explained adequately. There is, however, one marked exception. The implantation of the embryo is beautifully illustrated by the animation which alternates between two and three-dimensional views of the interior of the uterus and the inside of the blastocyst. This technique is most effective—why was it not maintained for perhaps another 10 minutes or so to surmount the hurdle of early tissue formation?

John Wilson

announcements**International meetings**

February 14, **Pesticides in Agricultural Control**, London (Mr J. M. Rowell, Society for Chemical Industry, 14 Belgrave Square, London SW1X 8PS, UK).

February 19–20, **Offshore Oil Needs Onshore Fabrication**, Glasgow (Colin Maynard, The Institute of Petroleum, 61 New Cavendish Street, London W1M 8AR, UK).

April 7–11, **Annual Chemical Congress**, York (Dr John F. Gibson, The Chemical Society, Burlington House, London W1V 0BN, UK).

April 9–11, **European Space Research Organisation Tribology Symposium**, Frascati, Italy (ESTEC, Mechanisms and Tribology Section, Structure Division, ESTEC, Domeinweg, Noordwijk, Netherlands).

April 23, **Machine Intelligence**, London (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1X 8QX, UK).

Miscellaneous

Residential course on enzyme and fermentation biotechnology. Organised by the Biochemistry Department at the University of Surrey and the Society of Chemical Industry, the course is aimed at young graduates and experienced workers in the field of technology. Further details from: Dr Alan Wiseman, Department of Biochemistry, University of Surrey, Guildford, UK.

Reports and Publications**Great Britain**

Chemistry and Industry Buyers' Guide 1975: Chemicals, Manufacturing Plant and Laboratory Equipment. Pp. 70. (London: The Society of Chemical Industry, 1974.) [2511]

Mycotoxins in Food. By Dr B. Jarvis. (Scientific and Technical Surveys No. 83.) Pp. 20. (Leatherhead: The British Food Manufacturing Industries Research Association, 1974.) [2611]

Department of the Environment. The Welsh Office. Report of a River Pollution Survey of England and Wales 1973. Vol. 3. Pp. xii + 32. (London: HMSO, 1974.) £1.20 net. [2711]

Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 277, No. 1269: On Finite Amplitude Convection in a Rotating Magnetic System. By P. H. Roberts and K. Stewartson. Pp. 287–315. (London: The Royal Society, 1974.) £1.05; £1.10 overseas. [2811]

Technology at Work. Pp. 34. (Cambridge: The Director—Corporate Projects, Pye of Cambridge, Ltd., 1974.) gratis. [2911]

People Without Choice. By Brian Abel-Smith. (Report of the 21st Anniversary Conference of the International Planned Parenthood Federation.) Pp. 68. (London: International Planned Parenthood Federation, 1974.) [2911]

Reshaping Britain: a Programme of Economic and Social Reform. (Broadsheet No. 658.) Pp. 98. (London: PEP/The Social Science Institute, 1974. Orders to Research Publications Services, Ltd., Victoria Hall, Fingal Street, East Greenwich, London, SE10.) £2. [212]

Natural Environment Research Council. Monks Wood Experimental Station—Report for 1972/1973. Pp. 103. (Abbots Ripton, Huntingdon: The Institute of Terrestrial Ecology, Monks Wood Experimental Station, 1974.) 60p. [212]

The Royal Society. Report of Council for the year ended 31 August 1974. Pp. 99. (London: The Royal Society, 1974.) [312]

The British Council. Annual Report 1973/1974. Pp. 107 + 10 photographs. (London: The British Council, 1974.) [312]

The Autonomy of the Broadcasters: Constitution and Convention. By Sir Michael Swann, FRS. Pp. 12. (London: BBC, 1974.) [412]

Science Research Council. Radio and Space Research, January 1971–December 1973. (The Report of the Director of the Appleton Laboratory.) Pp. 83. (London: HMSO, 1974.) £1.25. [412]

Parkinson's Disease. Pp. 24. (London: Office of Health Economics, 162 Regent Street, W1, 1974.) 25p. [512]

Forty-seventh Annual Report of the Agricultural Research Institute of Northern Ireland, 1973/1974. Pp. 47. (Hillsborough, Co. Down: Agricultural Research Institute of Northern Ireland, 1974.) [512]

CEGB Research, No. 1, December 1974. Pp. 36. (London: Central Electricity Generating Board, 1974.) [512]

Other countries

CERN—European Organization for Nuclear Research. CERN 74–20: Selected Physics Data on Neon-Hydrogen Mixtures. Compiled by H. Leutz, F. Schmeissner and H. Weninger. Pp. 175. CERN 74–21: *Radiation Measurements Around the Fermilab 3,000 GeV Main Accelerator*. By M. Awschalom, D. D. Yovanovitch, K. Goebel, K. P. Lambert, J. Ranft and E. Wilson. Pp. 35. (Geneva: CERN, 1974.) [1311]

World Health Organization. Technical Report Series No. 553: Ecology and Control of Rodents of Public Health Importance—Report of a WHO Scientific Group. Pp. 42. (Geneva: WHO; London, HMSO, 1974.) Sw.fr.5. [1311]

Smithsonian Contributions to Zoology. No. 173: *Ostracoda (Myodocopa) of Cape Cod Bay, Massachusetts*. By Louis S. Kornicker. Pp. 20. (Washington, DC: Smithsonian Institution Press, 1974. For sale by US Government Printing Office.) 65 cents. [1311]

Smithsonian Contributions to Zoology. No. 167: *Studies of Neotropical Caddisflies, XVII: The Genus *Smicridea* from North and Central America (Trichoptera: Hydropsychidae)*. By Oliver S. Flint, Jr. Pp. iii + 65. (Washington, DC: Smithsonian Institution Press, 1974. For sale by US Government Printing Office.) \$1.50. [1411]

Bulletin of the Museum of Comparative Zoology, Harvard University. Vol. 146, No. 3: *A Revision of the Cardinalfish genus *Epigonus* (Perciformes, Apogonidae), with Descriptions of Two New Species*. By Garry F. Mayer. Pp. 147–203. \$3.35. Vol. 146, No. 4: *The Spider Family Anyphaenidae in America North of Mexico*. By Norman Platnick. Pp. 205–266. \$3.25. (Cambridge, Mass.: Museum of Comparative Zoology, Harvard University, 1974.) [1811]

National Research Council Canada. NRCC No. 13688: Environmental Cause/Effect Phenomena Relating to Technological Development in the Canadian Arctic. By Wilson Eedy. (Associated Committee on Scientific Criteria for Environmental Quality.) Pp. 125. (Ottawa: National Research Council, 1974.) [1811]

CAS Today: Facts and Figures About Chemical Abstracts Service. Pp. 32. (Columbus, Ohio: Chemical Abstracts Service, A Division of the American Chemical Society, 1974.) [1811]

Glossary of Mental Disorders and Guide to Their Classification for Use in Conjunction with the International Classification of Diseases, 8th Revision. Pp. 86. (Geneva: World Health Organization; London: HMSO, 1974.) Sw.fr.12. [1811]

Publications of the Finnish Geodetic Institute. No. 77: *Beobachtungsergebnisse der Finnischen Winkelmessungen in Den Jahren 1969/1970*. Von Jussi Kaariainen. Pp. 40. No. 78: *High Precision Measurements for Studying the Secular Variation in Gravity in Finland*. By Aimo Kiviniemi. Pp. 68. (Helsinki: Geodeettinen Laitos, 1974.) [1811]

Recreation in a Marine Environment. Pp. 12. (Zurich: ICOMIA Marine Environment Committee, Gubelstrasse, 28, 1974.) [1911]

nature

February 6, 1975

Being circumspect about plutonium

PLUTONIUM has emerged into the public gaze again in the past year or so not only because of disquieting reports of lax security at places where it is stored in the United States but also because of fears that the radiation safety standards for the substance, in particular its radioactive isotope plutonium-239, do not properly take account of its true toxicity. The former problem could affect many people very quickly—for example if a terrorist group were to steal sufficient plutonium to cobble up a crude bomb—but it admits of a simple solution through improvements in security. The toxicity question is, however, a thorny one, which has implications, in the first instance, for those who work with plutonium, although the matter is by no means irrelevant to the public at large.

Unfortunately for the clarification of the issue, some of the statistics that seem to point to an even higher toxicity than previously thought are not agreed upon—notably those pertaining to the nuclear fuel reprocessing plant of British Nuclear Fuels Limited at Windscale on the north-west coast of England, where five employees, ex-employees and pensioners of the company (which was hived off from the United Kingdom Atomic Energy Authority three years ago) have died of leukaemia, according to company records, in the 24 years the plant has been operating. On the basis of an average 'risk population' of about 3,000 during that period, the company says that the expected natural incidence of leukaemia would be at least four, an indistinguishably different number statistically. Those who take issue with British Nuclear Fuels say that 3,000 is an overestimate, perhaps by a factor of three or more, of those actually at risk and, furthermore, that radiation workers who leave that kind of work altogether are lost from the system of medical monitoring and checks that operates in the nuclear industry (a situation which in itself strongly suggests the need for some sort of national register of people who have ever been radiation workers, perhaps like the Transuranium Registry in the USA).

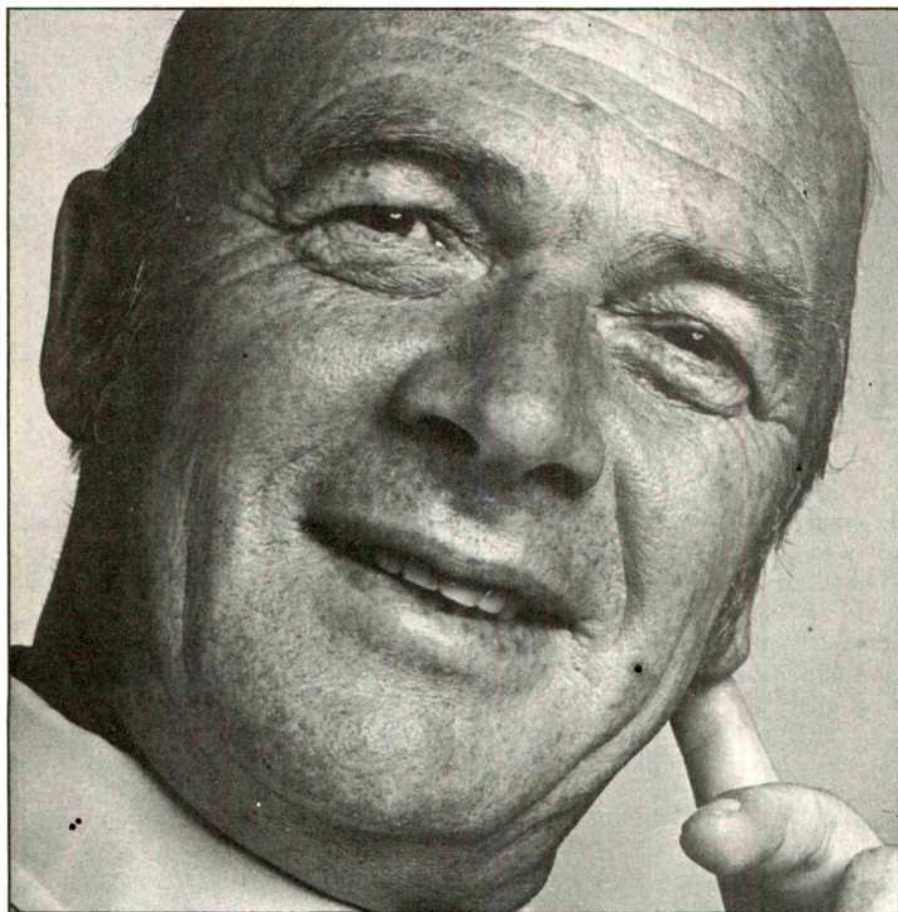
The statistics mentioned are at odds with each other to the extent that it has been argued that workers associated with plutonium are an order of magnitude more likely to contract leukaemia than people engaged in other occupations. Clearly, the only reasonable basis for new plutonium safety standards is a solid body of research and a careful analysis of the assumptions made in arriving at the present standards.

In a timely document published this week (*The Toxicity of Plutonium*, HMSO, 45p) the Medical Research Council (MRC), the body which advises the

government on radiation safety standards, taking into account the recommendations of the International Commission on Radiological Protection (ICRP), suggests that the part of the standard dealing with the inhalation of plutonium in insoluble form (for example, plutonium oxide, as used in the fuel of fast reactors) is too slack by a factor of five. The reasoning is based on the latest evidence available from the literature and on a reassessment of the 'critical organ' principle, which is still adopted for the plutonium standard by the ICRP, even though in the case of strontium-90 the commission has recognised that it is somewhat artificial. In essence, according to the principle, one regards a single organ or tissue as 'critical' in the sense that it is critically important in determining the MPAI (maximum permissible annual intake), at present set at 1 μ g on the basis that the lung is the critical organ. The MRC points out that several different organs—bone, liver and gut—are in fact "irradiated to a similar level of risk", and that in those circumstances a reduction in the MPAI would be required. The way the MRC's calculations come out, three of the MPAIs (those covering the ingestion of soluble and insoluble plutonium compounds and the inhalation of soluble ones) are about right, whereas the MPAI for inhalation of insoluble compounds is rather off-beam.

In its report, which contains a wealth of detail on all aspects of the toxicity of plutonium, the MRC also considers the 'hot particle' thesis, according to which the local irradiation of the lung by very active insoluble particles is markedly more carcinogenic than irradiation when the same activity is uniformly distributed. Although the report is unimpressed with the contention of Tamplin and Cochran that the MPAI for inhaled insoluble plutonium is 115,000 times too large for particles above a certain activity, this is a point which needs to be cleared up once and for all by experimentation on animals which are better models of human beings than beagle dogs. If work with, say, primates reveals a greater susceptibility to plutonium, then clearly the safety standards will have to be looked at again, and urgently.

No part of the MRC's report, of course, has a direct bearing on the way in which the standards are enforced or the steps taken to minimise accidents involving plutonium. In the UK the Nuclear Installations Inspectorate, together with the National Radiological Protection Board, is charged with that job, and the Royal Commission on Environmental Pollution is taking an overview of the whole situation in its examination of radiation hazards in general.



Vice-Chancellor Perry: "experience can be adapted"

Open for business

Britain's pioneering experiment in mass education, the Open University, has now developed to the point where it seems unlikely that any government will go so far as to close it down. This is a rather modest level of success—one would hardly be justified in saying that the Open University has in any sense 'come of age', even though it has produced its first graduates. But there was a real prospect that the incoming Conservative government of 1970 might have abandoned the experiment, and with higher education generally still in a parlous economic state the mere fact of the continued existence of the Open University is something to take note of. How might the bridgehead established by the Open University be used to best advantage in the coming years? And do conventional universities have something to learn from the Open University in terms of cost effectiveness?

—John Gribbin reports.

THE first lesson that might be learned by some academics is the combination of public relations ability and political awareness which the Open University staff seem to have. It's no secret that in the run up to the 1970 election the Open University's representatives took good care to lobby Margaret Thatcher, then in the Shadow Cabinet, and that this lobbying paid dividends when the Conservative government was elected, against all predictions, later that year. That might seem a wasteful diversion of effort which would, in an ideal world, be better spent on the development of the academic side of the university itself; but without that effort there might not

have been a university to develop.

Late in 1974, the Open University revealed to the public gaze an example of its business acumen with the establishment of a consultancy service "to help interested bodies overseas establish similar techniques and systems" to the teaching-at-a-distance techniques pioneered by the Open University itself. At that time, Sir Walter Perry, Vice-Chancellor of the Open University, commented that "in four years, the university has shown that distance teaching can be effectively deployed to solve pressing educational problems... There is now enough evidence to justify the belief that our experience can be successfully adapted to other environ-

ments and to other types of education", and a spokesman for the university stressed that although it has concentrated on teaching to degree level, "the Consultancy Service believes that similar techniques and systems can be applied to other kinds of education and training, especially life-long learning".

So the university is already looking well beyond the usual narrow confines of higher education establishments. This kind of facility could clearly be of great use in keeping teachers up to date, providing training in the law, or perhaps as a means of welfare and hygiene training. And these developments show clearly how the Open University might be made, if not to pay its own way entirely, at least to contribute substantially to its own running costs. The present marketing activities of the university are far from insignificant, as the table shows, although it must be born in mind that the "margin" mentioned there is not entirely profit—there are other overheads involved in running the marketing operation. The global scale of this marketing operation is also surprising to anyone used to thinking of the Open University as a British establishment; only just over 50% of present net sales are in the UK.

Perhaps the greatest "package" success of the Open University in the marketing field is the sale of complete courses to a few American colleges; in these packages books, course unit guides and broadcast material have been sold together and will be used just as they are in the Open University itself. This encourages speculation about the possibility of using modern communications facilities to centralise higher education to a great extent. If students in the USA can take what is in essence an Open University course (even, perhaps, an Open University degree) why should not the same facilities be used by UK universities? There is a powerful case to be made that, other considerations apart, the energy saving implicit in communicating a course to students, rather than transporting students to a course and housing them while they take the course, should alone lead to a major rethinking of our educational system. It is difficult to see that the interests of the country as a whole (or any country) would be less well served if the present scattering of scores of universities across the country were to be replaced by no more than half a dozen establishments organised along the lines of the Open University, perhaps on a regional basis. But that prospect is certainly not one which we are likely to see in the immediate future.

Any movement in that direction is bound to meet opposition from the academic establishment. But it is diffi-

grants, for example, is set to increase by almost \$40 million, to reach \$380 million.

As for the support of research and development in colleges and universities, an analysis provided by the Office of Management and Budget indicates that academic scientists will receive only about \$2,278 million of the total federal research budget proposed for next year. That represents an increase of only about \$52 million, which will not keep pace with inflation.

How will Congress treat the budget proposals? First, it should be noted that Congress this year has an entirely new mechanism for dealing with the federal budget. In the past, the Administration's budget proposals have been chewed over by individual appropriations committees in the House and the Senate, each of which is responsible for specific departments of the federal government. Since each committee operates independently, the process has been likened to each committee writing cheques without regard to what is in the bank.

This year, however, budget committees in the House and the Senate will establish an overall budget ceiling, and decide maximum amounts for each appropriations subcommittee to work to. Thus, for the first time, Congress will be able to compare directly the worthiness of programmes supported, for example, by NASA with those supported by the Department of Health, Education and Welfare.

Nevertheless, congress is unlikely to reduce budgets for such politically sensitive areas as energy research and development—although it may well re-order priorities within the energy research budget to favour environmental research, and solar energy and coal research, as against nuclear power. Similarly, the money available for biomedical research will probably be increased above the Administration's budget request.

Finally, it should be noted that two years ago, when Mr Nixon announced that he no longer required a full-time Science Adviser, the scientific community suffered a fit of apprehension that the federal science budget would immediately show a precipitous decline. But Dr H. Guyford Stever, who inherited some of the Science Adviser's responsibilities, noted last week that "some people have said that federal science has been drifting. I am pleased to note that it has been drifting upwards".

● During the past few years the National Institutes of Health (NIH) and its clients in the universities and medical schools have been awash with turmoil and indignation over the Administration's policies for supporting biomedical research. The budget un-

veiled this week will not help much in calming the troubled waters.

To begin with, the Administration has proposed cutting some \$315 million from the funds that Congress has already voted for the NIH for the 1975 fiscal year (which is now more than half over). Then, the budget proposed for the NIH in 1976 would reinstate only \$72 million of that cutback, to give the agency a total of \$1,805 million—\$279 million less than Congress has approved for 1975. To add insult to injury, the Administration is, naturally enough, claiming credit for proposing a \$72 million increase for NIH next year.

Another controversial item in the budget is that the Administration has signalled its intention to reduce expenditures on biomedical training. Attempts to do that in the past have invariably brought the medical schools to simmering point.

Although Congress will not go along with all the Administration's proposals for the NIH, another long and bitter battle can be anticipated and biomedical scientists will have to wait to see exactly how much money the federal government intends to spend on health research over the next 18 months.

As far as the NIH's budget for this year is concerned, the problem lies with an appropriations bill which Congress finally passed in December last year. Although the bill exceeded the funding levels recommended earlier by President Ford for a number of programmes, Ford signed the measure into law with the understanding that he would later ask Congress to revise it. His proposed revisions include reducing the total which Congress appropriated for the National Cancer Institute by \$128 million, and similarly taking \$38 million from the budget of the National Heart and Lung Institute and axing a total of \$190 million from the budgets of the other eight institutes. Furthermore, he has proposed slicing \$104 million from the funds that Congress has appropriated for programmes dealing with alcoholism, drug abuse and mental health.

Congress must, however, give its express approval within 45 days before any of those reductions can be made, and it is not likely to do so. For one thing, Congress raised up the NIH's budget in December in spite of an urgent plea from Ford to reduce it. And for another, during the past four years Congress has consistently appropriated much more money for biomedical research than the Administration has wanted to spend. It is conceivable, however, that, as a token gesture, Congress may shave a little off the NIH's budget for this year just to show willing to hold the line on federal expenditure.

To put the matter in perspective,

however, it should be noted that even with the proposed revisions in the NIH's 1975 budget, the agency would still have some \$265 million more to spend this year than last.

Thus, with budgets for the 1975 fiscal year far from settled, the Administration's proposals for next year are totally unreliable as a guide to what will actually be spent. Nevertheless, for what it is worth, the Administration has proposed a budget of \$605 million for the National Cancer Institute and \$293 million for the National Heart and Lung Institute. Those proposals would represent increases of \$36 million and \$7 million respectively over the revised budgets of the two agencies, neither of which is sufficient to keep pace with inflation, and both of which are well below the budgets approved by Congress for this year. A similar picture can be painted for all the other institutes of NIH.

As for biomedical training, the Administration has proposed that the NIH should allocate some \$124 million to training grants next year, compared with \$131 million this year. That proposal is unlikely, however, to find much sympathy either in Congress or in the medical schools, for last summer Congress passed a bill which authorised expenditures of some \$208 million a year on biomedical training. Congress approved that measure in response to previous attempts by the Administration to do away with the NIH's training programme entirely—attempts which threw the biomedical research community into apoplexy.

Thus, the federal government's support of health research is again marked by considerable confusion and is likely to become a source of yet more controversy. But two factors may help to clarify the situation in the next few months.

First, Senator Edward M. Kennedy has announced that he will carry out a thorough investigation of the federal government's biomedical research policies this year through his Senate Health Subcommittee. Although Kennedy has usually supported the biomedical research community's complaints against the government, he served notice in a speech at Yale University last month that the medical colleges have often failed to examine critically their most cherished programmes. And last week, President Ford finally appointed five members of a special commission, set up by an Act of Congress, which will undertake a review of the NIH's policies and programmes. The review will take 18 months and co-chairmen of the panel are Dr Franklin Murphy, chairman of the board of the *Los Angeles Times*, and Dr Robert Ebert of Harvard University. □

AN OPEN letter from the Soviet dissident Dr Andrei Sakharov to the head of KGB, Mr Y. V. Andropov, has just reached the West. The letter (shortened slightly in this version) reads as follows:

'On December 20, I received a letter from a mythical Russian Christian Party, which contained threats directed at my son-in-law, Efrem Yankelevich, and my one-year-old grandson.

The authors of the letter threaten to destroy them, if I continue my civic activity.

The content and whole tone of this letter make it obvious that it was composed by your officials with the aim of frightening me and forcing me to keep quiet.

The previous day I had received notification from the visa office that Yankelevich and his wife had been refused permission for a journey to the United States at the invitation of the President of the Massachusetts Institute of Technology.

Prior to this, their application had lain unanswered for a year and eight months. This coincidence cannot be accidental. I declare that the members of my family are hostages and are being used as a means of exerting pressure on me.

This has been confirmed again today. Two of your officials, who were tailing my son-in-law on the street, repeated word for word the same threats as before (adding some foul language) and demanding an end to my activity.

I demand an end to the harassment of me. I demand an end to the thugery, and assurances of security for my family.

Don't disgrace your department even more by threatening the lives of children in the way practised in Stalin's time.

In the present circumstances, for which your department bears the responsibility, I demand that permission be granted at once to Efrem Yankelevich, his wife Tanya Semyonova and their son to travel to the US for an unlimited period, but on Soviet passports so that they will have the right to return home when their security can once again be assured.

It is quite clear that they are hostages. The only way of ending this form of pressure on me is to give them visas at once.

I also demand immediate permission for my wife to travel to Italy for treatment (for her blindness).

I demand an end to the disconnecting of my international phone calls.

I demand an end to the judicial and extra-judicial persecution of my friends, including Sergei Kovalov, who was arrested on 27 December.

At the same time as sending you this letter I am making it generally available and appealing to the world public for its support and defence.'

Vera Rich adds:

With the enforced departure of Solzhenitsyn and Medvedev, the Academician Andrei Sakharov, has found himself in an increasingly exposed and lonely position as Russia's "voice of conscience". That the Soviet authorities would like to silence him was only too apparent from the vicious press campaign launched against him in September 1973. This having failed, the alternatives remaining are exile or

cation for emigration, began on a large scale in 1970-71, long before the top-level trade talks began. He says that the idea of limited emigration was decided at the Politbureau meetings held in preparation for the Twenty-fourth Party Congress which opened on March 26, 1971. (A large party of emigrants left on March 1st). It would seem, however, that the scale of the applications somewhat disconcerted the Soviet authorities, resulting in the imposition for a time of the "education tax" on Jewish intellectuals wishing to emigrate, together with the policy of harassment and general unpleasantness which persists to this day.

For the future, Polskii sees the continuation of "limited" emigration, but with increasing pressure on applicants, especially intellectuals. The five cities of Moscow, Leningrad, Kiev, Khar'kov and Minsk together account for 1,000,000 Jews—some 30% of the Jewish population of the Soviet Union, and virtually the entire Jewish intelligentsia. It is from these cities, that he expects emigration to be most difficult. Further, there is the concept of the "closed town", which has been mentioned in visa refusals—towns such as Sverdlovsk, from which, it appears, no emigration is to be permitted at all.

The current pressure on *refusniks*—dismissal from one's job, followed by prosecution for parasitism (being without visible means of support), may well account for a certain falling off in the number of applications. Nevertheless, says Polskii, the would-be emigrants are not entirely abandoning hope. While not actually putting in a visa application at present, a great number of them are careful to keep themselves provided with an up-to-date invitation from a relative in Israel, so as to be ready to apply the moment circumstances seem slightly more propitious.

● The Russian biologist and writer Vladimir Konstantinovich Bukovskii, at present serving a twelve-year prison sentence is a result of supplying Western psychiatrists with documents on the confinement of dissidents in mental institutions is reported to be dangerously ill. Although suffering from a heart condition related to rheumatism, a liver condition, and duodenal ulcers, Bukovskii is receiving no medical attention, and medicines sent to him by his mother have been returned. He is unable to digest the prison diet of salt herring, spratts and cabbage. In an appeal to Minister of the Interior Sholokov, sent on January 29, Nina Bukovskaya pointed out that her son "is being slowly murdered by being deprived of medical treatment and correct medical diet". To date, no reply to her appeal has been received.

Dissident voices



Polskii: expert *refusnik*

physical violence—directed not at himself but at his family—his wife, stepdaughter and her husband and baby.

● Physicist Viktor Polskii, who, after more than four years' delay, was finally allowed to leave the Soviet Union for Israel in December 1974, is perhaps one of the best authorities on the problems facing the "refusnik", since in his case, the customary harassment included a charge of dangerous driving, which could have carried a considerable prison sentence, but which, under the pressure of world opinion finally resulted in a fine of 100 roubles (£50).

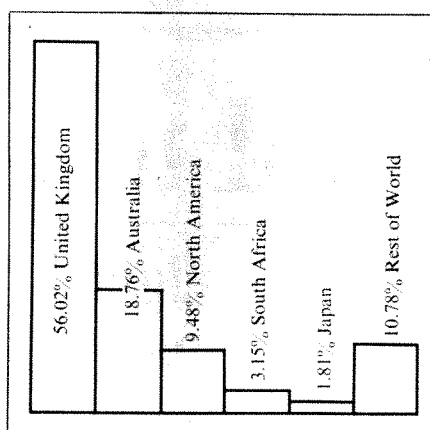
Talking to *Nature* this week about Jewish emigration, and, in particular, the emigration of Jewish scientists from the Soviet Union as a result of the recent rejection of the trade agreement, Polskii stressed that in fact the policy of permitting limited emigration to Israel was begun before the Soviets entered upon their course of *detente* and trade agreements with the USA. Emigration, or rather, the appli-

cult to counter the argument that the Open University approach is simply more efficient at getting its message across to large numbers of people at reasonable cost. The BBC runs just one studio for Open University programmes, at Alexandra Palace in London, and each television programme takes about one day of studio time to produce. There is also an outside broadcast unit, and radio programmes seem to be produced with hardly any effort at all. With about 50 producers at Alexandra Palace who have some kind of science qualification and this rather shoe-string operation the unit produces programmes which are used for five years before being replaced. And although the initial cost may seem high, the cost per student over that period must compare favourably with the cost per student of conventional lectures, even before account is taken of sales of programmes to other educational establishments.

In addition, for many of the science programmes the demonstrations provided in the Open University programmes are far more elaborate than anything which could be put on, and expected to work, under the restrictions of an ordinary lecture theatre. Again, an experiment which could not be financed by dozens of colleagues separately each academic year is a viable proposition when it only has to be put on once for the benefit of five years' intake of a much larger student body.

Even so, the total cost of the Alexandra Palace operation is not excessive even by the standards of some 'ordinary' universities, and looks even more reasonable when set against typical government expenditure on education and science. The total number of people now working in this BBC department is just over 300, and in 1973-74 the cost of the operation (wages, transmissions, filming, graphics, scenery, rental, rates, lines, administration, travel, processing and presentation all included) was £2,090,000. About half of this goes on wages, £600,000 for the actual manufacture of radio and television programmes (including all repeat fees), £250,000 for transmitter running costs and some £200,000 for premises (including lighting, heating and so on).

With a smaller proportion of pupils in secondary schools in Britain now wanting to stay on to take A levels, and fewer of those who qualify at that level wanting to continue their education at university level immediately afterwards, perhaps the time has come to rethink our approach to higher education. The arguments in favour of a break between school and university are well known, and perhaps the Open University approach offers the best route by which



O.U. sales, nine months to Sep. '74.

to return to the academic system for prospective students who had, temporarily at least, had enough of studying and examinations by the time they had reached A-level standard. Without yet going so far as to reduce the number of conventional universities drastically, it does seem that flexibility in combining Open University education with more conventional courses could make the university system as a whole more efficient, both in terms of cost and in terms of providing the greatest flexibility of courses for the greatest number of students.

The longer courses offered by conventional establishments are obvious possibilities for this kind of development; the time is not yet ripe for the Open University to offer a complete medical course (if only because of the difficulties in obtaining practical experience) but is there any real reason why all prospective medical students should not complete a 'remote teaching' pre-medical course before moving on to practical work? It is also difficult to see why law could not prove an ideal

course for the Open University treatment, if only the lawyers could be persuaded to move into the last quarter of the twentieth century. And if pre-medical courses are feasible, why not 'pre-university' courses, either as an option or as an essential prerequisite for all students who wish to take degrees? Two years of such study, say, would provide an excellent opportunity both for the prospective degree student to decide if he really does want to return to the academic fold and for the university of his choice to decide if they really want him. It would not be unreasonable for the work involved in such a two-year period of study to count as one third of a degree course, or, following the present Open University practice, for the same workload to be spread over three or four years. Then, the period of residence at traditional university need only be two years, with considerable advantages for the efficient running of those establishments. As most universities that run 'mature student' schemes can report, there would probably be great advantages in terms of a reduction in the drop-out rate and an improvement in academic standards.

There are imperfections in such a scheme, as there are in most, and it would hit hard at some entrenched positions. But with the present problems of the educational system (which have recently led the Secretary of State for Education and Science, Reg Prentice, to order what has been called "an inquiry on the reluctant students") something must not only be done but must be seen to be done. In the absence of other constructive proposals, this possible line of development of the Open University must surely be considered seriously. □

Open University marketing:
ANALYSIS OF SALES FOR NINE MONTHS ENDED SEPTEMBER 30, 1974

Item	Quantity	Value £	Cost £	Margin £
Book	168,149	177,347	67,710	109,637
Tape	3,550	20,642	6,840	13,802
Film	2,253	91,940	54,072	37,868
Home experiment kit	16	1,933	1,440	493
Colorimeter	45	1,026	828	198
Microscope	401	6,759	3,609	3,150
Sound level indicator	245	5,435	1,813	3,622
Logic tutor kit	97	1,983	839	1,144
Analogue control kit	45	586	256	330
Power supply meter	34	664	383	281
Bobcat	103	537	304	233
Gramophone record	221	228	110	118
		309,080	138,204	170,876
Other income				
Royalties—Harper and Row		8,000	—	8,000
Other		4,070	—	4,070
Film library		8,702	1,803	6,899
Recording licences		2,810	—	2,810
		332,662	140,007	192,655
Sources of total sales:				
UK		£181,000		
Overseas		£152,000		
		£333,000		

international news

THE economic theories of John Maynard Keynes, coupled with the rising price of oil and the insatiable demands of the Pentagon, have helped produce promises of massive increases in federal support for science and technology in the United States. That, at least, is the prospect held out by President Ford's budget proposals for the 1976 fiscal year, which were unveiled earlier this week.

Taken at face value, the budget anticipates that the federal government will set aside some \$21,600 million for research and development next year—a huge increase amounting to \$2,822 million above the expected support for science and technology this year. At 15.5%, the increase would be more than enough to keep pace with inflation and if it is all spent, it would represent much the biggest increase in the science budget since the Second World War.

Unfortunately, however, the figures cannot be taken at face value and should be treated with extreme caution. For one thing, they will almost certainly be altered by Congress and, for another, the picture is not quite as rosy as it seems at first sight.

The President's budget is a remarkable document. Hundreds of pages of facts and figures richly laced with rhetoric, it is the product of thousands of man-years of effort and as such it is the most important policy document produced by the Administration each year.

The sharp increases which have been proposed for research and development next year owe their existence to three chief factors. The first is a massive increase in the budget proposed for military science and technology. The second is another sharp upturn in proposed

US budget: energy and the military do well

from Colin Norman, Washington

spending on energy research and development, and the third is the fact that President Ford, like his predecessor, has been converted to the Keynesian view that an expanding federal budget is a useful weapon against recession. Ford's total budget would entail outlays of \$350,000 million and a budget deficit of nearly \$52,000 million.

● A total of \$11,400 million has been proposed for military research and development, which represents a staggering increase of \$1,900 million over anticipated expenditures this year. The budget document is characteristically vague about how this increase in funds will be spent, but the Trident submarine, the B-1 strategic bomber and a project to provide "options for possible future deployment of a new intercontinental ballistic missile system" are among the items mentioned. In addition, a large increase has been proposed in a programme for testing nuclear weapons, the objective being to complete all large-scale testing before the introduction of a possible threshold test ban. Some \$201 million has been requested for nuclear testing.

The Department of Defense's share of the proposed military research and development budget amounts to some \$10,600 million, of which just over \$2,000 million is classed as research.

● Energy research and development is set to receive an increase of nearly

\$200 million, to reach a total of \$2,360 million next year. (See table).

Although nuclear fission would get the bulk of those proposed funds, and the liquid metal fast breeder reactor alone is set to receive nearly \$500 million, massive increases have been proposed for solar, geothermal, fusion and coal conversion technologies. The Energy Research and Development Administration have also announced plans to build a large Tokamak fast reactor at Princeton University in 1977. Designed to burn deuterium and tritium the machine would be the first fusion fast reactor in the US and it is expected to cost \$215 million.

● The Administration has proposed an increase of nearly \$300 million in the budget of the National Aeronautics and Space Administration, chiefly for the development of the Space Shuttle.

Simple addition shows that between them, those three areas carry off all but about \$480 million of the entire increase proposed for the federal science and technology budget. Some programmes have therefore been squeezed.

Biomedical research is one such area, and space research is another. No new space project will be started by NASA next year, and aside from development of the Shuttle the budget for most NASA activities will be held constant. Sufficient money has, however, been proposed to keep all existing space projects alive, and NASA has also been allowed to proceed with plans for a third in the series of Earth resources technology satellites.

Nevertheless, the overall picture for science and technology is remarkably bright. According to an analysis prepared by the Federal Council on Science and Technology, the budget would entail an increase of about 8% in expenditures on basic research, thereby reversing the trend of declining support of such activities which began in the early 1970s. Between 1970 and 1974, for example, federal outlays for basic research declined by 15% in purchasing power.

Another indicator of proposed support for basic research is the fact that the budget for the National Science Foundation (NSF) is set for a healthy increase. Overall, the Administration has proposed that the NSF's budget should increase by \$78.3 million next year, to reach a total of \$775.4 million. Some 83% of the NSF's budget is earmarked for support of basic research. The NSF's support through individual

Federal energy research and development programme (\$ million)			
Programme area	1974	Obligations 1975*	1976*
Direct energy research and development:			
Fossil energy	110	435	440
Solar and geothermal	45	102	123
Conservation	39	86	88
Nuclear energy	756	942	1,102
Environmental control	66	103	83
Total direct	1,016	1,669	1,837
Support programme:			
Environmental effects	138	264	273
Basic research	175	233	250
• Total support	313	497	523

*Estimate.

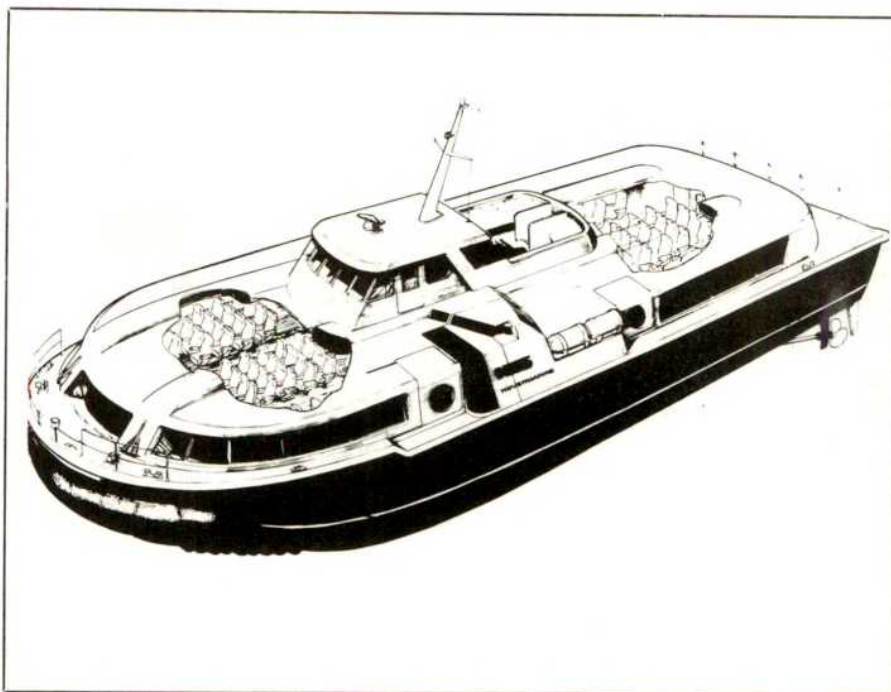
Making money and influencing people

from Angela Croome

LAST week the UK National Research Development Corporation (NRDC) announced that it was putting slightly over £500,000 into the development of a 200-passenger sidewall hover-ferry, the Hovermarine 5 (HM5). This caused some raised eyebrows, as Hovermarine, managed by Britons with a factory at Southampton, is now an American-owned company and the NRDC is, as its name suggests, dedicated to the promotion of inventions and applications to the national benefit using government money.

The loan is not anomalous, however, though it is of more than average interest. The NRDC holds the key hovercraft patents and issues the licenses for using them worldwide, so that any expansion by their licensees (such as Hovermarine) brings cash flowing in to London. (The NRDC itself has made a profit for the last few years.) In fact under its new American-style management Hovermarine's craft are now more widely sold than any other hover-ferries in the world, with 30 HM2s operating in 14 different countries, and the business has consistently doubled each year since the 1969 takeover. The company has played down the "new technology" aspect of the product and has emphasised its solid commercial and environmental advantages—such as its high speed, low cost, wake-free passage and high manoeuvrability. Currently, Hovermarine is the only licensee for sidewall hovercraft. Sidewalls, which provide solid containment for the air cushion along the craft's long axis and, acting as twin keels, aid manoeuvrability, essentially economise on fuel and power compared with competitive craft such as the fully amphibious hovercraft, the motorboat and the hydrofoil. The HM2 cruises at 30 knots; the HM5 is expected to cruise at 45 knots. By putting up the most substantial sum it has spent on hovercraft for a number of years the NRDC is saying in unequivocal fashion that it is pinning much faith in the future of sidewall hovercraft as sheltered-water ferries and, by implication, approving the commercial and sales skills of its licensee every bit as much as its technical flair.

The NRDC has just passed its 25th anniversary and has also gained a new Managing Director, Bill Makinson; he is not new to the corporation, however, having worked there since the mid-1960s. Does the major new loan reflect a shift in policy with a change of team, or the fruit of a quarter of a century's experience in seeking out and fostering worthwhile innovations?



The Hovermarine 5: £500,000 development programme

On the occasion of the 25th anniversary and the announcement of the annual accounts, the NRDC revealed that it was being heavily underused and described efforts to rectify the position. It is entitled to borrow £50 million from the government for encouraging promising inventions and techniques. Only £21 million is at present in use. The board is concerned that the existence of the corporation, much less its role and method of operation, is scarcely known to the part of the population that could benefit from it. Government research establishments have provided a steady stream of ideas for NRDC sponsorship in the past. These are now being deflected to some degree, following the rearrangements resulting from the Rothschild customer-contractor principle. Only 2% of private inventions that reach the corporation are worth following up. Few small and medium businesses—a major untapped source of innovative concepts—seem to be aware of the NRDC's existence. Good ideas from the universities are even rarer and this is attributed again to ignorance of the existence of the corporation. The NRDC has over the past couple of years found that there is not only a total shrinkage of good proposals coming its way but that the balance is poor. It likes to keep a reasonable match between its nine groupings.

In the face of this surprising situation in a tight money market, the corporation has launched an advertising campaign to get its opportunities better known. It has also set aside £1 million specifically for investment in good ideas from the universities. There is no indication that either move has had much impact.

There are various criticisms that can be levelled at the NRDC's present mode of operation and which may well be bound up with its faceless image. Just how it runs, and on what basis decisions are reached, are obscure. Just how much interference it experiences or fears from government departments is an open question. For instance is it really likely that a proposal turned down by Science Research Council would be pursued by the NRDC—the Laithwaite 'magnetic river' project is a case in point. The corporation claims that Whitehall has never gibbed at one of its projects; but is that necessarily a matter for congratulations? The virtue of such a body is that it should provide a genuinely alternative option. There is a widespread sense that the corporation operates too much as a bank and insufficiently as a sponsor of research and development as its title suggests it should. It is free to encourage and invest beyond the development phase of bringing a good product to the market place, and it is now generally acknowledged that the major costs arise at the production and marketing stage. But there are few, if any, cases where the NRDC has helped over these final and most costly hurdles, and plenty of examples of products that have failed because of lack of capital or expertise at the end of the 'run'.

There is no lack of expertise, skill and sound judgment in NRDC. It just does not seem to make the most of itself and under present economic conditions there is surely more need of such a supporting body than there ever was. The stocktaking at the start of its second quarter century seems an excellent moment for reassessment and review. □

THE underlying purpose of the annual meeting of the American Association for the Advancement of Science (AAAS) is not all that easy to pin down. Partly an attempt to improve that ill-defined entity the public understanding of science, and partly a forum for exchanging views on a variety of social and political issues, it is, in the words of the retiring AAAS President, Roger Revelle, a "scientific smorgasbord". Whatever its *raison d'être*, the 141st such event took place last week in New York City and a good time was had by all.

The theme of the meeting was "Science and the Quality of Life", which was interpreted broadly enough to include a session on the emotional reactions of plants and a talk by Isaac Asimov on "The Science Fiction Writer as Prophet". Discussions concerning the health effects of pollutants, the energy crisis, world food shortages and urban technology constituted a large part of the programme and there was even the occasional session devoted to scientific research.

One notable feature of the meeting was that few of the sessions were devoted to discussion of scientific results, undiluted—or unenriched as the case may be—by considerations of public policy. That aspect reflects the desire on the part of the organisers to make the annual AAAS meeting more concerned with the interactions of science and society. It also reflects the fact that few scientists think of saving up their research results to announce them at the AAAS meeting—more specialised gatherings and quick-publication journals now fulfil that role.

● With Vice-President Rockefeller engaged in a study of how science advice should be plugged into presidential decisions, a session devoted to the question "Does the President still need a science adviser?" may seem like the height of irrelevance. But it turned out to be highly pertinent. Although only one member of a panel, consisting chiefly of luminaries from the world of science policy, ventured the opinion that the President has no need for a science policy office at his elbow, there turned out to be a huge spread of opinion on what the mechanism should be, and on what it should do.

Before the meeting took place, a few of the participants held a press conference at which two former officials in the Nixon White House confirmed what most people had suspected—that Nixon abolished the post of science adviser because he disagreed with much of the advice he was getting. Dr Edward David, Nixon's last science adviser, spoke of a "falling out"

between the President and the scientific community and said that "there definitely was a perception in the White House that the science advisers were using their expertise to grind axes". And Clay Whitehead, former Director of the White House Office of Telecommunications Policy, said that White House officials felt that the science adviser showed more allegiance to the scientific community than to the Administration.

New York diary

from Colin Norman

Most members of the panel thought that an office to provide policy analysis and advice on issues involving science and technology should be relocated near the centre of power. Franklin Long, of Cornell University, a former member of the President's Science Advisory Committee (which Nixon also abolished), favoured a three-member council of science and technology advisers. David also argued for a science policy office, but warned that it should be established in such a way that it does not upset the existing (and little noted) arrangements for getting science advice into the National Security Council and the Domestic Council. But George Reedy, former press secretary to President Johnson, argued that a full-time science adviser is not needed and that an *ad hoc* arrangement to provide scientific analysis of specific issues for the White House would be a better arrangement.

● A little light entertainment was provided later in the week by a debate—if a piece of comic opera can be so termed—over whether or not plants have been shown to respond emotionally to external stimuli. The debate took place between a number of botanists and Mr Cleve Backster, a polygraph expert and former CIA employee, who carried out an experiment in 1966 which, he claims, shows that plants respond electrically to human thoughts and emotions. That experiment has since turned up as the foundation of a best-selling book, the *Secret Life of Plants*, and resulted in widespread popular belief in the United States that plants have emotions.

Backster, an ebullient man who displayed a strong tendency to lead with his chin, failed to convince many of his critics that his experiment demonstrated anything other than poor scientific method, while two scientists who had tried unsuccessfully to repeat Backster's amazing work, failed to shake the belief of about half of the

audience in the emotional capacity of plants.

The crucial experiment, published in the Winter 1968 issue of the *International Journal of Parapsychology*, was carried out in Backster's New York laboratory. It was an attempt, in short, to see whether plants respond electrically to the termination of animal life.

He hooked three plants up to polygraphs, by sandwiching one of their leaves between electrodes, and monitored their electrical activity when brine shrimp were killed in a separate room. The shrimp were killed (terminated, Backster says in his paper), by being dumped into a bath of simmering water. Amazingly enough, the polygraph chart registered electrical responses similar to those which show up when human emotional reactions are monitored, and the results were statistically highly significant.

But two other scientists related their fruitless attempts to repeat Backster's work. Edgar L. Gasteiger, of Cornell University, told how experiments conducted over two years by three of his students "with many types of control that Cleve Backster did not use" failed to show any plant response to the killing of brine shrimp. The other would-be repeater of Backster's results was John Kmetz of the Science Unlimited Research Foundation—a body which Backster himself helped to establish. Kmetz used a total of 42 plants, 84 shrimp drops and 84 control drops using sterile water. His plants failed to react.

Although he has not repeated his original experiment, Backster, undeterred, claims to have achieved even more spectacular results with the bacteria in yoghurt. He claimed that when he added milk to one yoghurt culture, another culture across the laboratory which was hooked up to a polygraph gave off electrical signals. That piece of information, not surprisingly, got widespread attention from the press, for, as one journalist put it, "whose editor can resist a story about yoghurt that talks?"

Perhaps the chief question raised by the session was why was it held. Dr Arthur Galston, of Yale University, who arranged it, said that a poll of his students turned up the finding that about half of them talk to their plants, and about half of that group believes that it does some good. "There is a gap", he said, "between what scientists believe and what the lay public believes", and it is important to give the issue an airing. It must be the first time that an entire session of the AAAS has been devoted to a single experiment which has not been repeated in eight years, even by its originator.

news and views

RECENTLY our knowledge of the recipes which galaxies might use for cooking up heavy elements has increased rapidly. In an article in the *Astrophysical Journal* (193, 327; 1974) Peimbert and Torres-Peimbert report the detection of a difference of helium abundance between the interstellar gas in our own Galaxy, as observed in the Orion and η Carina nebulae, and ionised gas regions in the Large Magellanic Cloud, one of the nearest neighbours to our Galaxy. The difference is small (about 20%), but this is the first time a difference has been detected which is significantly greater than the observational and interpretive errors. Although the bulk of helium in the Universe is thought to have been synthesised in the big bang, further enrichment with helium comes through the nuclear conversion of hydrogen as the primary energy source for stars. The low helium abundance observed in the Large Magellanic Cloud implies that the gas in that galaxy has not been enriched as much as that in our own Galaxy. The present conditions in the Universe suggest that the big bang would have produced isotopes of hydrogen and helium, but no metals. The metals (which to an astronomer are anything heavier than helium) come from thermonuclear reactions during supernova explosions of stars which die with a bang rather than a whimper, the metals observed in the interstellar medium probably originating from stars with mass of more than ten solar masses. The low helium abundance of the Large Magellanic Cloud fits in very well with the observed low metal abundance for the same system—again this shows less enrichment by material processed in stars.

The determining factor in the amount of enrichment that takes place almost certainly involves the ratio of mass in stars to the mass in gas which is set up during the early fragmentation (or perhaps agglomeration?), collapse and evolution of a protogalaxy into a galaxy from the debris of the big bang. The rather irregular Magellanic Clouds have considerably more gas than our spiral Galaxy, in accord with the general observation of a rough inverse correlation between the metal abundance and gas content of galaxies. Just when and how many stars form (perhaps determined by the amount of angular momentum present), together with how much gas is left, will not only affect chemical evolution but may

Galactic cookery

from M. G. Edmunds

also determine the morphological type of the galaxy on the Hubble sequence—the reason why chemical evolution is of interest to all studies of evolution and dynamics of galaxies.

A first approach to models of chemical evolution, examined in some detail by Tinsley (*Astrophys. J.*, 192, 629; 1974), comes through observation of stars in our own Galaxy. It has been known for many years that the simple idea of stars forming, evolving and exploding at a uniform rate is inadequate. The metal abundances of stars can be deduced from spectroscopic analysis and their ages deduced by comparing their spectroscopic and photometric properties with theoretical stellar evolution models. The resulting relation of metal abundance with age shows puzzling features. Enrichment in metals has certainly not been uniform, but seems to rise rapidly during the early part of the Galactic life and to have increased only very slowly since then. Even some of the oldest stars seem to be metal rich. A second problem occurs because it is observed that the number of metal-poor dwarf stars is far too small compared with the number of metal-rich dwarfs to be explained by uniform enrichment. So what really happened? A popular proposal is that early on (perhaps even before the Galaxy had formed) the relative number of high mass stars to low mass stars formed was greater than for recent star formation. This shift in mass function, caused either by forming more massive stars or fewer less massive stars, implies more supernova metal synthesis per mass of gas converted into stars, and a rapid build-up of elements with only a few dwarfs formed before the Galactic gas became quite rich in metals. A change in the initial mass function for star formation is not as arbitrary as it might seem because the initially low metal abundance could radically alter both the cooling of the gas required for protostellar collapse and the mechanisms which limit the greatest mass which can condense into a star. The biggest uncertainty in all

models of galactic evolution arises because we just do not have a reliable theory of star formation—we have no way of predicting even whether a low metal abundance would shift the mass function towards higher or lower masses.

Playing around with the star formation in a rather different way has produced the attractive “metal-enhanced star formation” model of Talbot and Arnett (the latest in a series of papers appeared in *Astrophys. J.*, 190, 605; 1974). They propose that star formation at a given time only occurs in regions of gas which are more metal-rich than average, although there is some uncertainty whether or not large enough inhomogeneities in the metal abundance of the interstellar medium can be set up. Metals are preferentially incorporated into stars, the model accounts for the observed abundance versus time variations and the metal-poor dwarf problem, and has the additional attraction that a constant initial mass function is perfectly adequate. If this model is correct, it is telling us interesting things about star formation—the requirement for high metals and an invariable mass function.

In external galaxies, and probably our own, another phenomenon requires explanation. The abundance of metals is greater near the centre of a galaxy than farther out. How were such abundance gradients set up? Larson (*Mon. Not. R. astr. Soc.*, 166, 585; 1974) and Larson and Tinsley (*Astrophys. J.* 192, 293; 1974) have investigated numerically the collapse of a spherical protogalactic gas cloud with various boundary conditions, and simple assumptions about the conditions for star formation. Some of these models reproduce well the time variation of metal abundance, and also set up abundance gradients by the fall of gas, enriched by supernova explosions, towards the centre of the galaxy. Another feature is the prediction of an early peak in star formation and explosion which Larson suggests might trigger the onset of Seyfert or QSO behaviour in some massive galaxies. This has attractions as it suggests a quiescent ‘normal’ evolution of a QSO until it becomes superluminous, and would allow the build-up during the normal evolution of the observed (albeit rather uncertain!) approximately solar abundance of metals in quasar spectra. In his most recent paper (*Mon. Not. R.*

astr. Soc., **169**, 229; 1974) Larson adds a new refinement to his models in the effect of the energy input of the supernovae on the galactic gas. This energy tends to blow out of the galaxy the metal enriched gas as it falls towards the centre, the amount expelled depending on the escape velocity and hence the mass of the galaxy. This offers an explanation of the apparent correlation of the metal abundance of a galaxy with its absolute magnitude, from several times less than solar abundance for dwarf ellipticals with low escape velocities to a few times more than solar for massive ellipticals.

A rather imposing blend of an initial formation model and a collapse-infall model is being proposed by Ostriker and Thuan. From other arguments on galactic rotation curves and stability of galactic disks, Ostriker and co-workers have proposed that spiral galaxies have massive stellar holes containing perhaps up to ten times the mass of the disk. During initial collapse star formation is rapid, and the en-

riched gas expelled from massive stars falls to form the disk. The longer lived dwarf stars persist in the halo, sufficiently faint to escape visual detection, but still contribute a significant infall of material into the disk by mass loss from their surfaces. For all models the realistic inclusion of such effects as violent events in the nuclei of galaxies, sporadic star formation and radial gas flows is yet to come. But already we have the problem of non-uniqueness of models which will only be resolved by tighter theoretical and observational constraints. For example the useful limits on the amount of gas cycled through star formation from deuterium abundances (which is destroyed by passage through stars) can be used once it is clear whether or not the big bang was the only source of deuterium.

It seems that present galactic chemical evolution models are similar to culinary ones—they may vary greatly in their ingredients, but with judicious stirring a palatable cake may result from many of them.

The enigmatic protein

from Aubrey Knowles

It is nearly 100 years since Kuhne extracted a pink, light-sensitive material from the retinas of a number of animals. He realised that these substances—later called rhodopsins—were proteins and that they were responsible for the conversion of light into neural signals. Since then, a great deal of effort has been made to understand the rhodopsin molecule, but its structure is as mysterious as ever. This unique molecule is thermally quite stable, yet will undergo major chemical and structural changes on the absorption of a single quantum of visible light: beyond acting as a transducer of light, it is also the principal structural protein of the photoreceptor cell membranes.

This last factor has hampered investigation by the usual techniques of protein chemistry, for rhodopsin is a hydrophobic molecule and is totally insoluble—thus it cannot be crystallised. Until recently, most investigations have been carried out on detergent extracts, and the results of these are of questionable validity for all detergents denature the molecule to some extent. The detergent-extracted molecule has been found to contain, beyond the protein part, phospholipid, carbohydrate and a polyene called retinal. The latter is the 'chromophoric group' that gives the molecule its colour: exposure to light causes the chromophoric group to break away from its binding site, and the colour is lost. The supposition that the binding site is on

the protein part of the molecule has recently been confirmed, for nearly all of the phospholipid can be removed enzymatically without loss of colour (Borggreven, Daemen and Bonting, *Archs Biochem. Biophys.*, **151**, 1; 1972). It has also been found that up to 40% of the protein can be removed by proteolysis without causing bleaching (Bonting, de Grip, Rotmans and Daemen, *Expl Eye Res.*, **18**, 77; 1974; Trayhurn, Mandel and Virmaux, *FEBS Lett.*, **38**, 351; 1974; Saari, *J. Cell Biol.*, **63**, 480; 1974).

In 1968, Heller (*Biochemistry*, **7**, 2906 and 2914) showed that rhodopsin protein can be separated by gel filtration chromatography, and that it behaves as a globular protein of molecular weight about 28,000 and diameter 46 Å. The retinal molecule has a molecular weight of only one-hundredth of that of the protein, but since it is about 15 Å long, if it is visualised as fitting into a binding site on the surface of the protein, it will span a considerable arc of the circumference. In fact, Saari claims to have removed many of the hydrophilic groups of the protein, which apparently lie on the surface, without disturbing the chromophoric group, and so the binding site may well be a cleft in the interior of the protein. In either case, the chromophoric group will lie in close proximity to a large number of the amino acid residues of the protein—close enough for electrostatic or dispersive forces to act between them.

Absorption maxima

It is this interaction that gives the rhodopsin molecule its unique spectroscopic properties. All known creatures with image-forming visual systems employ a rhodopsin-type visual pigment, and the absorption maxima of these pigments lie over a colossal range of wavelengths throughout the visible and near-ultraviolet regions. The gamut of rhodopsins has been extended down to 345 nm by the confirmation by Paulsen and Schwemer (*Biochim. biophys. Acta*, **283**, 520; 1972) that the pigment of the neuropteran, *Ascalaphus macaronius*, is a true rhodopsin. The retinal-based pigment of longest wavelength is that of the bronze turkey (*Meleagris* sp.), with a maximum at 562 nm. Thus any theory of pigment absorption must explain how the absorption maximum of retinal at about 380 nm can be either blue-shifted by 35 nm or red-shifted by up to 182 nm. Various theoretical models have been put forward in the past, but the most popular at present is based on a single covalent bond—a protonated aldimine (Schiff's base) linkage—between retinal and an ε-lysine group on the protein. This will cause the retinal absorption maximum to be red-shifted by up to 100 nm, and the fine tuning required to give the absorption maxima of different species is then achieved by secondary interactions between the chromophoric group and other amino acids. This can be pictured in its simplest form as the effect of a single negative charge fixed at a certain point near the polyene chain. Recent detailed molecular orbital calculations show that as this counter-charge is moved away from the positive charge at the aldimine bond, the absorption maximum goes to longer wavelengths, approaching a limiting value similar to that found in nature (Waleh and Ingraham, *Archs Biochem. Biophys.*, **156**, 261; 1973; Suzuki, Komatsu and Kitajima, *J. Phys. Soc. Japan*, **37**, 177; 1974).

Retinal can form a number of geometrical isomers, but only one of these, the 11-*cis*-isomer, will react with bleached rhodopsin to regenerate the original pigment. For this reason, it has been assumed that the conformation of the chromophoric group in the binding site is 11-*cis*, and that the shape of the molecule is the same as is found in crystalline 11-*cis*-retinal. Gillardi, Karle and Karle (*Acta Cryst.*, **28B**, 2605; 1972) showed that crystalline 11-*cis*-retinal is not planar like the other isomers, but is twisted and should thus be optically active. The intact rhodopsin molecule shows a circular dichroism associated with the long wavelength absorption band that is not seen in solutions of 11-*cis*-retinal. Honig, Kahn and Ebrey (*Biochemistry*, **12**, 1637;

WHETHER the study of the orientation of ancient monuments ranks as a science or a pseudo-science remains a matter for debate, but there can be no doubt that the topic is once again attracting a great deal of attention. Having formed a focus of interest in the latter years of the nineteenth century and early in the twentieth century, studies of orientation went into decline in the inter-war years partly, perhaps, because of factors such as excessive emphasis on allegedly significant orientations in Nazi Germany, and partly because the different groups of people involved—mainly astronomers and archaeologists—had failed to agree on the reality and significance of the results.

The recent revival of interest in orientation studies has arisen mainly from new detailed surveys of megalithic sites in the UK, especially those carried out by professor Thom since his retirement from Oxford. He has used these measurements in two ways—to identify the astronomical events indicated by the orientations and to consider the actual layout and scale of the monuments. The consensus of opinion at a recent joint meeting of the Royal Society and the British Academy on ancient astronomy tended to be that the deductions concerning astronomical orientation had firmer bases than those relating to the existence of a standard measure of length in megalithic times. Certainly the former have excited the more discussion. For, unlike most archaeological discoveries which illuminate primarily material culture, they could give a direct, if partial, insight into the level of intellectual sophistication that had then been reached.

Astronomers have in general contributed to the recent growth of interest in studies of alignments more by theoretical speculation than by on-site surveying. Indeed, much of the controversy engendered by the claimed alignments has hinged on the alleged extravagances of some of these

Ancient observatories

from A J Meadows

speculations (especially regarding the astronomical implications of Stonehenge). The crucial point behind both the speculations and the arguments hinges on the significance of any measured orientation. After all, astronomical objects have to rise and set somewhere on the horizon—could not the observed alignments be purely coincidental? How convincing an answer can be given to this question depends critically on the accuracy and uniqueness of the data collected.

A group from the Cambridge University Astronomical Society has now re-surveyed with increased accuracy three sites previously described by Thom (this issue of *Nature*, page 431). The most interesting of the three sites is that at Ballochroy in Argyllshire (which Thom has described as one of the most important he knows for observing the solstitial Sun). The new measurements not only support Thom's claim, they also produce a set of dates for the various orientations present that agree remarkably well among themselves, and convincingly suggest that the stones were erected for use around the date 1600 BC. But the other two sites surveyed—at Loch Seil and Loch Nell, both also in Argyllshire—provide much less satisfactory evidence. At Loch Seil, the suggested alignments seem to be non-existent, and at Loch Nell, although the alignments are clearly defined, they do not point to any obviously significant astronomical event.

The sceptic might reasonably comment that one interpretable site out of three suggests that chance may well play a part in producing apparently significant orientations. But chance

would be unlikely to produce orientations that agree with respect to dating, as do those found at Ballochroy. Besides, disturbances must certainly have occurred at some sites during the past four millennia, disrupting alignments that were originally there, and making interpretations ambiguous. The interesting discrepancies are rather those, as at Loch Nell, where the intended alignment seems obvious, but the astronomical explanation does not, for this raises the question whether all, or even the majority of, megalithic monuments were really intended to serve as astronomical observatories. One comment is worth making on this: there is still a need to consider all astronomical bodies that might have attracted attention in megalithic times. So far, attention has concentrated mainly on the Sun, Moon and a few bright stars. However, the planets, especially Venus, were surely equally important in ancient astronomy. The problem, in terms of orientation studies, lies in distinguishing between solar and planetary positions on the horizon.

If we compare studies carried out in recent years with those occurring earlier in the century, the only apparent difference in practical terms is an increase in the accuracy and extent of the site surveys. Why then has the subject re-erupted into the news? I would guess that one reason is a new pervasive interest in the influence of astronomical (or astrological) ideas on the thinking of early man. This is reflected in recent discussions of the astronomical implications of myth, noticeably the detailed analysis in *Hamlet's Mill* by G. de Santillana and H. von Dechend. It appears, too, in the debate over very early archaeological material that, according to A. Marshack, indicates a primitive lunar notation. All these topics are controversial, but, in combination, they suggest the intriguing conclusion that man was numerate long before he was literate.

1973) have suggested that this is due to the stereospecificity of the binding site, which can select one enantiomer from a solution of 11-*cis*-retinal, and thus confer optical activity on the visible absorption. On the other hand, Johnston and Zand (*Biochemistry*, **12**, 4637, 1973) have shown that model aldimines of all-*trans*-retinal with some optically active amines show circular dichroism, so long as the amine has an aromatic group that can couple with the long-wavelength absorption band of the retinal. Thus the fact that rhodopsin shows circular dichroism in

the visible region implies only that the chromophoric group is bound to an optically active amino acid, and that there are aromatic amino acids near the binding site. The retinal is not necessarily in a dissymmetric conformation.

Intact retina

Another physical method of great potential in probing the structures of coloured molecules in biological milieux is the resonance-enhanced Raman effect. In 1970, Rimai, Kilponen and Gill (*Biochem biophys Res Commun*, **41**, 492, 1970) showed that using this

technique it is possible to study the chromophoric groups of rhodopsin molecules in the intact retina, and thus provided the first direct evidence that the retinal is bound in the form of an aldimine. The intense 488 nm exciting light used in these experiments bleached the rhodopsin, but it has since been shown that use of a 583 nm dye laser permits the recording of the Raman spectrum without undue bleaching, and so it could be established that the chromophoric group is probably in the 11-*cis* conformation (Lewis, Fager and Abrahamson, *J Raman Spect*, **1**, 465,

1973) Unexpected bands in the 550–950 cm^{-1} region are ascribed to interactions of aromatic amino acids with the chromophoric group—perhaps these are the same interactions that determine the absorption maximum of the pigment. Confirmation that the aldimine linkage of rhodopsin *in situ* in photoreceptor membranes is protonated has recently been presented by Oseroff and Callender (*Biochemistry*, **13**, 4243, 1974), who also studied the effect of light on the pigment at low temperatures. Illumination gives an equilibrium mixture of rhodopsin and two products, isorhodopsin and bathorhodopsin, which are all interconvertible by light. The conversion of rhodopsin to isorhodopsin requires isomerisation of the chromophoric group from the 11-*cis* to the 9-*cis* form, and the conversion to bathorhodopsin was previously thought to involve isomerisation to the all-*trans* form. However, Oseroff and Callender conclude that the retinal in bathorhodopsin is not in any conformation known from studies of solutions. Bathorhodopsin is the first of a series of intermediates seen in the bleaching of rhodopsin at normal temperatures; the absorption maxima of these lie between 543 and 380 nm, and it is tempting to think that these represent stages in the twisting of the chromophoric group as it progressively breaks free of the binding site. Resonance Raman spectroscopy promises to be a splendid tool in the study of these intermediates, and may also explain the mechanism that determines the absorption maxima of the rhodopsins from different species.

Another component of the rhodopsin molecule that is in the news is the carbohydrate moiety, originally identified by Heller and Lawrence (*Biochemistry*, **9**, 864, 1970) who suggested that it acts as a marker on the surface of the molecule to ensure its correct orientation on assembly into the photoreceptor membrane. Renthal, Steinemann and Stryer (*Exp Eye Res*, **17**, 511, 1973) have since established that it is not involved in the binding site, for chemical modification of the carbohydrate does not prevent the regeneration of bleached pigment. The group also provides a convenient point of attachment for a 'fluorescent probe' molecule, and a study of energy transfer between the probe and the chromophoric group suggests that these are separated by between 51 and 65 Å—more than the apparent diameter of the protein. This may mean that the rhodopsin molecule is ellipsoidal, or could be explained by the recent studies of Romhányi and Molnar (*Nature*, **249**, 486, 1974) who have shown by light-microscope histological methods that the saccharide chains of the rhodopsin molecules in fixed frog receptors point

outwards from the molecule. It is suggested that these extend into a hydrophilic region in the receptor membrane, and serve not only to anchor the molecule in a fixed orientation but also to act as a pivot for its rotation. Surprisingly, Saari reports that proteolysis does not remove the carbohydrate moiety, even though this degradation is thought to involve the hydrophilic parts of the molecule most remote from the chromophoric group.

We are in an exciting era of visual pigment research, when many of the old dogmas are being re-examined and new physical techniques now permit measurements to be made on the behaviour of rhodopsin in intact photoreceptor cells. Perhaps we shall soon have a real insight into the structure—and finally the function—of this strange molecule.

Basin tectonics

from A Hallam

It is no discredit to the elegant simplicity of plate tectonics that only in the oceanic sector does it throw significant new light on vertical (as opposed to lateral) motions of the Earth's surface. Plate tectonics revolutionised the study of structural evolution by accounting for major tectonic features of the Earth by comparatively few lateral motions and interactions of lithospheric plates. Not surprisingly, the success of the new concept pushed into the background related problems which have been wrestled with since the time of Lyell and Darwin—problems associated with the uplift of continental mountain ranges and plateaux and the subsidence of basins or continental margins. In the preparation of dishes for the connoisseurs of world tectonics we seem to have become obsessed with plates and neglected basins.

The widespread indications of marginal downwarping of continents such as seaward descent of erosion surfaces and warping of marine terraces, seaward dip and thickening of Cretaceous and Tertiary sediments, palaeogeographical evidence of founded offshore land areas and, more controversially, submarine canyons, led many geologists to agree with Kuenen's judgement of a quarter century ago that it was "difficult to avoid the conclusion that marginal areas have subsided to form part of the present deep sea floor". Now isostatic considerations demand that this could only happen if the continental crust were substantially thinned or in some mysterious way converted into denser oceanic-type crust. The Soviet tectonician Belousov has gone so far as to invoke extensive 'oceanisation' of continental crust to

account for the ocean basins, he has in consequence totally rejected plate tectonics and explains first order structures exclusively by vertical motions. One need not go so far as to adopt this extreme heretical position to accept that something strange and as yet inadequately explained has been going on at continental margins.

Subsidence of parts of the North Atlantic continental margins has recently been shown by geophysical work and deep sea drilling to be even more extensive than recognised hitherto. Thus Talwani and Eldholm (*Bull Geol Soc Am*, **83**, 3575, 1972) have demonstrated that the Voring Plateau off Norway, occupying an average depth of 2,000 m, is subsided continental rather than oceanic crust, with an eastward-descending ridge of probably Precambrian basement buried beneath a considerable thickness of Palaeozoic and younger sediments, it can thus be compared with, for instance, the Rockall Bank. East of Newfoundland is another area of deep sea floor which gives indications of being subsided continent (Ruffman and van Hinte, *Geol Surv Can Paper* 71–23, 407, 1973). Drilling on the Orphan Knoll seamount has located Middle Jurassic continental coal measure deposits, which points indubitably to over 2,000 m of subsidence since that time. Ruffman and van Hinte argue for a much more extensive subsidence, of as much as 3,500 m, of a vast area of deep sea floor to the south and west of Orphan Knoll.

This work has some interesting implications. It suggests for instance that the so-called Quiet Magnetic Zone is not, as most geophysicists have assumed, true oceanic crust produced by spreading at a time of no magnetic reversals, but subsided continental margin, as I inferred a few years ago (*J Geol*, **79**, 129, 1971). This would explain why the position of the Quiet Magnetic Zone is always near the continental margin irrespective of the postulated age of oceanic opening. Second, the common acceptance of the 1,000 m isobath as the effective edge of the continents for the purpose of least squares fits, to obtain their pre-drift positions, is seen to be inaccurate and leads to their being placed too close together. This could well account for the most awkward feature of the celebrated Bullard fit of the Atlantic continents, namely the lack of space for Central America. In terms of plate theory the marginal downwarping can be explained as follows. Prior to or contemporary with initial oceanic rifting the continental crust was bowed up by expanding hot mantle and thinned slightly by erosion but more substantially by tensional stretching accommodated by normal faulting. As the continents subsequently moved apart

Hexokinase in human muscular dystrophy

from a Correspondent

CONTROVERSY abounds at the moment about the pathogenesis of the severe X-linked form of muscular dystrophy, which is known as the Duchenne type and which usually results in the affected boys being unable to walk by the age of about 10 years and rarely surviving beyond the age of 20. Within recent years McComas, Sica and Currie (*Nature*, **226**, 1263, 1970) have suggested that the progressive degeneration of skeletal muscle which occurs in this condition may be the result of a primary defect in the motor neurones, whereas Hathaway, Engel and Zellweger (*Archs Neurol*, *Chicago*, **22**, 365, 1970) have postulated a process of progressive ischaemia of muscle because of a superficial similarity of the pathological changes in skeletal muscle in such cases to those produced by experimental muscle embolism in animals. These hypotheses have challenged the traditional view that this and other forms of genetically determined muscular dystrophy are likely to be due to a primary biochemical abnormality of the muscle cell itself, though it is certainly possible that a genetically determined biochemical defect could influence the function of both motor neurones and skeletal muscle.

Ellis, Strickland and Eccleston (*Clin Sci*, **44**, 321, 1973) have previously found that muscle obtained by biopsy from patients with Duchenne type muscular dystrophy is less able than is normal muscle to utilise glucose by the usual pathway of glycolysis. They found that in such tissue there was partial conversion of glucose to fructose instead of to glucose 6-phosphate under certain metabolic conditions. The

conversion of glucose to glucose 6-phosphate is normally catalysed by hexokinase, an enzyme present in mammalian tissue in the form of several isoenzymes which differ in their K_m values for glucose.

Strickland and Ellis have now found certain abnormalities of hexokinase isoenzyme mobility when studied electrophoretically in muscle obtained from patients suffering from X-linked Duchenne type muscular dystrophy and the similar but more benign X-linked Becker variety (this issue of *Nature*, page 464). They find that tissue obtained from patients with these two diseases shows three, instead of the normal two, bands for hexokinase, two of which move relatively faster than do the bands of the normal isoenzyme II, and an additional slower-moving band close to isoenzyme I. Similar isoenzyme changes were found in liver, brain and sciatic nerve obtained at post-mortem from patients with Duchenne type muscular dystrophy. As the authors point out, this alteration in hexokinase isoenzyme II in a number of tissues obtained from patients with muscular dystrophy might account for a restricted flow of glucose through the hexokinase step in tissues where isoenzyme II is required. If such an abnormality were to prove to be of fundamental importance in the pathogenesis of muscular dystrophy, it could certainly account for abnormalities in the function of both neurones and muscle cells in this disease. Confirmation of this work and further studies of hexokinase isoenzyme behaviour in muscle and other tissues obtained from patients with X-linked muscular dystrophy will be awaited with interest.

the trailing margins continued to subside as the newly created ocean floor cooled progressively and hence became more dense. It remains to be seen whether this model can account in a satisfactory quantitative way for several thousand metres of subsidence since the early Cretaceous, apparently regardless of the age of opening.

Although the Atlantic margins offer interesting problems their solution seems relatively straightforward compared with those posed by substantially land-locked marine basins such as the Gulf of Mexico, the Black Sea and parts of the Mediterranean Sea. For the Black Sea Brinkmann (*Am Ass Petrol Geol Mem*, **20**, 63, 1974) has

outlined the abundant palaeogeographical evidence that it was the site of a terrestrial sediment source for much of the Palaeozoic and early Mesozoic. Yet seismic surveys reveal that the crust underlying the Black Sea has suboceanic thicknesses. Probably the most plausible interpretation of the existing data is that thick Upper Cretaceous and Tertiary sediments rest on a much attenuated continental crust. The obvious question arises, what has caused the thinning? Unlike the case of the Atlantic margins it cannot readily be related to a generally accepted plate tectonic model.

The case of the Mediterranean basins is perhaps even more intriguing, since

we have two quite different interpretations of their origin, one invoking lateral, the other vertical movements. Western Mediterranean basins such as the Balearic Basin and Tyrrhenian Sea have a crust which is at least suboceanic if not fully oceanic in character, though no magnetic anomalies indicative of seafloor spreading have been discovered. Palaeomagnetic evidence for the anticlockwise rotation of Corsica, Sardinia and Italy has been held to confirm Carey's proposal that the western Mediterranean basins are sphenochasms, that is, wedge-shaped sectors of ocean floor created by the movement apart of slabs of continent. Hsu and others have adopted this model to account for the existence in the deep basins of substantial deposits of late Miocene NaCl and CaSO₄ salts which bear the characteristics of continental salina and sabhka deposits. To explain this apparent paradox a boldly imaginative hypothesis has been put forward (Hsu, *Nature*, **233**, 44, 1971; Hsu *et al*, *Init, Rep Deep Sea Drilling Project*, vol 13, 1203, 1972). After Africa closed on Europe during the early Tertiary and sphenochasms opened up in the intermediate zone, a stage was reached when a series of land-locked basins several thousand metres deep were created in the Miocene. Because the climate was warm and arid the Mediterranean remained substantially dry, except for shallow brine pools in the deep basins, where salts began to precipitate. River canyons were cut down as much as 2,000 m through the surrounding continental margins to a base level well below ocean level. Subsequently, in the Pliocene, the Straits of Gibraltar were opened up and sea water flooded in from the Atlantic, rapidly drowning the basins and canyons and allowing normal deep sea muds to be deposited. As a defence against an admittedly extravagant, though attractive, hypothesis, Hsu has quoted Sherlock Holmes: "When you have excluded the impossible, whatever remains, however improbable, must be the truth."

But can the 'impossible' be excluded?

Let us consider some other facts. There is an old idea that the Tyrrhenian Sea, like the Black Sea, is the site of a subsidised landmass. This idea has received impressive support from the dredging of continental metamorphic rocks from the margin of a tilted fault block in the centre of the sea, implying late Tertiary subsidence in excess of 3,000 m (Heezen *et al*, *Nature*, **229**, 327, 1971). The occurrence of a parallel series of ridges and troughs suggests a series of fault blocks like the Great Basin regime of the western United States and implies considerable thinning of continental crust by tension. More recently, combined geophysical

and stratigraphical work in the Balearic Basin and the margins of Catalonia has provided strong support for substantial subsidence in the recent geological past (Stanley *et al*, *Geology*, 345, July 1974). Prominent stratigraphical horizons of Miocene or younger age have been demonstrated to be downwarped or down-faulted towards the basin floor by as much as 2,000 m. The submarine canyons evidently result from the drowning of previously emergent land margins to depths of more than 1,000 m. In other words the Miocene salt was originally deposited at or slightly above sea level and has subsequently subsided with the underlying crust.

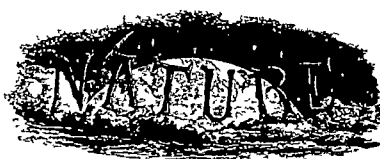
One of these explanations must be wrong. If we are to adhere to Hsu's hypothesis how are we to account for the apparently good evidence of basinal subsidence? If accordingly we reject it we must presumably also reject the palaeomagnetic evidence for the rotation of Corsica, Sardinia and Italy and must also try to explain up to 3,000 m subsidence in the last 15 million years, and the substantial loss of continental crust. Is there any correlation between the basin subsidence and the uplift of certain circum-Mediterranean mountain areas? To give just one instance, locally in Tunisia there has been at least 3,000 m uplift since the late Miocene. If the crust has been thinned by tension this must presumably have operated radially, as also would be the case in the Gulf of Mexico, which similarly has oceanic crustal structure, buried salt (probably Jurassic) in its deepest part and suggestions from palaeogeography of occupying the site of an ancient landmass. To what extent are the pronounced changes in elevation a consequence of changes in crustal thickness or density, or variations in density of the upper mantle? Can the underlying phenomena be readily reconciled with our present geophysical concepts, which lean heavily on plate tectonics, or are other mysterious forces at work?

My dear Watson, it's not so elementary!

Oceanic ridges as collapse calderas

from Peter J. Smith

THERE are obvious differences, both in structure and behaviour, between the long linear oceanic ridge and the geographically localised volcano. But there are also significant similarities, for in their active forms both features are associated with very young volcanic rocks, rocks which have undergone high temperature metamorphism and high heat flow. Moreover, some volcanoes produce lavas similar to the basalts found on the ocean floor. These



A hundred years ago

THE words of Mr Disraeli on Monday night with regard to University Reform are also very cheering to those who wish to see some decided action taken towards the thorough reform of our Universities. Mr Disraeli's words were very strong, so strong indeed as to amount to an assurance that Government really means to take into serious consideration this session the Report of the University Commission. "It is our opinion," the Prime Minister said, "that no Government can exist which for a moment maintains that the consideration of University Reform, and consequently legislation of some kind, will not form part of its duty." These words give out no uncertain sound. Mr Disraeli said, moreover, that when the Report was presented at the end of last session, the Colleges were not assembled. It would be interesting to know whether the Colleges have yet met to consider the Report, and whether they are likely to act on this hint of the Premier and take some internal action—commence the work of reform from within, instead of waiting until they are driven to it by forces from without.

from *Nature*, 11, 292, February 11, 1875

characteristics suggest that ridge axes may be underlain by magma chambers at a depth of a few kilometres. Is it possible, then, that the study of volcanoes with magma chambers will help in the understanding of the less accessible ridges?

In the hope that such a comparison would be illuminating, Francis (*Geophys J*, 39, 301, 1974) has analysed the activity of the Fernandina Caldera (Galapagos Islands), whose floor collapsed in 1968. In the early part of that year, Isla Fernandina, a large basaltic shield volcano, had an elliptical caldera with a flat floor about 7 km² in area. But on June 11 the caldera floor began to subside in a series of short bursts and along a steep pre-existing elliptical boundary fault. According to Simkin and Howard (*Science*, 169, 429, 1970), the increase in caldera volume caused by the collapse was 1–2 km³, and the lowering of the floor (unevenly) by an average of 150 m represented a potential energy loss of 10²³–10²⁴ erg.

The seismic energy released was several orders of magnitude lower than this (10¹⁹–10²¹ erg), but its effect was hardly less spectacular. Whereas in 1967 and 1969 earthquakes were reported from the area at an average rate of about one every two months, at

least 599 shocks occurred during the period 12–23 June, 1968. The largest of these events, most of which were shallow, had maximum magnitudes $m_b=5.4$ and $M_s=5.2$. But more significantly, the magnitude–frequency plot, whether using m_b or M_s , is best fitted by two straight lines rather than one; there is a definite change in slope (b value) at about $m_b=4.9$ or $M_s=4.5$.

The two distinct b values for M_s data were first recognised by Filson *et al* (*J Geophys Res*, 78, 8591, 1973), who were, however, unable to explain them. But Francis has now been able to extend the Filson model to give a straightforward explanation of the seismic data. The caldera floor is regarded as the top of a plug capping a magma chamber (plug and chamber being cylindrical in the simplest form of the model). The fact that the Fernandina caldera collapsed along a pre-existing fault surface implies that under normal circumstances the plug is not supported structurally but by the pressure of the magma beneath. A collapse will occur when, for one reason or another, the magma chamber pressure is reduced to the point at which the shear stress on the fault surface exceeds the fault strength. When this happens, the plug will fall to a new equilibrium position.

From the model, and using a modified form of Press's (1967) formula relating fault length to earthquake magnitude, Francis shows that the maximum magnitude of shocks produced along the main caldera fault should be about 5.4 (m_b). This agrees with observation. But a model involving a falling rigid plug is not entirely satisfactory insofar as, in the real Fernandina collapse, further faulting occurred at the north-west end of the caldera. Here there was no appreciable subsidence, and flexing of the caldera floor produced a band of fractures aligned normal to the caldera's long axis. The length of this fracture zone is an order of magnitude smaller than that of the main fault and the predicted maximum magnitude of shocks from the zone is correspondingly low at $M_s \sim 4.0$.

Further calculation shows that the larger shocks produced at the main fault are associated with low average stresses and small stress drops—which implies high b values. By contrast, the tensional fracturing in the north-west has stresses comparable to the average stresses for most earthquakes, the b values are thus also comparable (normal). In other words, the two distinct b values observed for the Fernandina collapse swarm arise from two different types of earthquake.

So the seismic characteristics of the Fernandina caldera collapse are broadly explicable in terms of observed physical processes. But how does this relate to oceanic ridges? The point that

Francis makes is that in many respects the seismicity of slow-spreading ridges (fast spreading ridges such as the East Pacific rise are rather different) is similar to that of the caldera collapse episode. At the mid-Atlantic ridge, for example, all earthquakes are shallow and none is very large (maximum $m_b \sim 5.6$ in rift zone), rift zone earthquakes seem to be confined to the median valley, the b value of rift zone events is usually significantly higher than for transform fault events, rift zone events exhibit normal faulting, and earthquakes swarms are common along rift zones. Moreover, earthquake swarms associated with spreading axes in the Gulf of California have been shown to be low stress events.

Francis thus proposes that rift zone seismicity along slow-spreading ridges is generated by a series of caldera collapses. The median valley floor of the mid-Atlantic ridge, for example, is constantly collapsing at various points, the locations of which will be governed by the configuration of the magma chambers and of the overlying topography. Generally, collapse will occur along pre-existing faults, although when such faults have moved outside the influence of the magma chamber (by lithospheric spreading) new faults will form along the chamber's lateral limits. The width of the median valley floor thus roughly defines the maximum width of the magma chamber.

Where is the myosin?

from Dennis Bray

THE history of muscle research would have been very different if striated muscle had not been built in such a well ordered fashion. The almost crystalline myofibrils, composed of parallel filaments in segmented arrays, first caught the eye of light microscopists and later provided material for electron microscopy and X-ray diffraction. The information they provided on the positions and mode of interaction of the two kinds of elemental filament was unique and independent of biochemical analyses of their component proteins, such as actin or myosin.

Curiously, we are now in precisely the opposite position with non-muscle cells. Most eukaryotic cells have been shown to contain proteins that are chemically very similar to actin and myosin and which probably work in the same way, at the molecular level, as their counterparts in muscle. But, with the exception of the bundles of actin-like filaments that some cells possess close to their membrane surfaces, we have little idea where these proteins are within the cell. How they might be linked to other cellular constituents, or produce coordinated movements, are still open questions.

Some of the difficulties in placing these proteins within the cell are illustrated by the variety of results recently reported for myosin. The myosin of blood platelets and cultured fibroblasts seems to be more soluble than muscle myosin, and does not condense so readily into thick filaments at physiological ionic strength. If these cells are disrupted and subcellular fractions prepared by differential centrifugation (in the now time-honoured way) then most of the myosin is found in a soluble, non-sedimentable form (Ostlund, Pastan and Adelstein, *J. biol. Chem.*, **249**, 3903, 1974). To judge from this direct and quantitative method most of the myosin is freely diffusing within the cell, either as single molecules or as small aggregates.

Yet if a different technique is used a different answer is obtained. The recent work of Weber and Groschel-Stewart (*Proc. natn. Acad. Sci. U.S.A.*, **71**, 4561, 1974), in which cultured fibroblasts are fixed and exposed to anti-serum to uterine myosin, shows that at least part of this protein is to be found in filaments. The striking pictures they obtained by immunofluorescence show long straight strands extending across the cell, often in parallel alignment, and with a periodicity in the staining revealed at higher magnification. Since there is some reason to think that these strands may also contain actin an arrangement similar to that in myofibrils may be present, and it is tempting to think that such strands may be able to contract.

And the possibilities do not end there. Again using immunofluorescent techniques but this time applying them to intact cells, two laboratories have reported the presence of myosin on the outer surfaces of cells (Gwynn *et al.*, *J. Cell Sci.*, **15**, 279, 1974; Willingham *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **71**, 4144, 1974). Conscious, perhaps, of the unexpected nature of their findings these workers have carried out extensive controls. Willingham and co-workers in particular, compared antisera to three types of myosin: fibroblast, smooth muscle and striated muscle, and found that only the first of these gave the surface reaction. Among other tests, they carried out the important one of absorbing anti-serum to the cells and showing that upon elution it still cross reacted with myosin.

It is true that immunological evidence in the absence of direct biochemical tests is hard to defend against all objections. Myosin, in particular, is a difficult protein to purify and few would claim that their preparation could not contain up to 5% of other components, perhaps from the surface. In addition, the possibility of adventitious contamination by myosin from

lysed cells is always present. Nevertheless, an interaction of contractile proteins with cellular membranes is now widely expected and could have as a consequence the exposure of part of the myosin molecule to the outside of the cell. It will be most exciting if this is true, and worth the search for independent methods of testing.

The present picture seems confusing. We have evidence that myosin is free within the cytoplasm, present in strand-like filaments, and closely associated with the cellular membrane. But these are not necessarily incompatible results. Only one of the methods used to detect myosin is quantitative—that of measuring its ATPase activity—and the immunological results could apply to only a small fraction of the myosin within the cell. It is entirely conceivable that this protein could exist in more than one state of aggregation or be associated with more than one subcellular structure. If so, then the factors that control its distribution will necessarily be critical for the movements of the cell.

Proline implicated in halophyte osmotic adjustment

from Peter D. Moore

JUST as adaptations to cold and to heat seem to go hand in hand (*Nature*, **253**, 11, 1975), so do adaptations in many plants to drought and salinity. Water-stressed plants of several species (for example broad bean, Stewart *et al.*, *Plant Physiol.*, **41**, 1585, 1966, and barley, Singh *et al.*, *Nature new Biol.*, **236**, 188, 1972) have shown an accumulation of the free amino acid proline. Singh *et al.* showed that different varieties of barley had different capacities for the accumulation of proline in response to water stress.

Waldren and Teare (*Plant and Soil*, **40**, 689, 1974) have now examined proline levels in intact plants of sorghum and soybean and at the same time measured stomatal diffusive resistance and leaf water potential as indications of water stress. In soybean, proline only began to accumulate when the stomatal diffusive resistance exceeded 37 s cm^{-1} and when a leaf water potential of -14 bar was reached. By this time the plants showed loss of turgor. In the case of sorghum, proline accumulation occurred only when stomata were completely closed and when the leaf water potential had reached -24 bar. Again, incipient wilt was evident at this stage, so proline concentrations cannot be regarded as a sensitive indication of water stress for these species.

This type of association of free proline with drought response in plants

has led Stewart and Lee (*Planta*, **120**, 279, 1974) to investigate the possibility that proline may be associated with the tolerance of physiological drought in halophytes. They analysed the free amino acid content of a number of halophytes from salt marshes in western Britain and found that in many (for example *Aster tripolium*, *Armeria maritima*, *Puccinellia maritima* and *Triglochin maritima*) the concentration of proline was higher than that of any other single amino acid, often by a factor of ten. In some species proline represented 70% of the free amino acids and in most it exceeded 30%. But *Plantago maritima* had a low proline content, this exception demonstrates that proline accumulation is not a universal feature of halophytes.

Using *Triglochin maritima* Stewart and Lee were able to show that proline build-up is enhanced by high salinity in the surroundings, just as Waldren and Teare had demonstrated in the case of water stress. They also found that different populations of certain species, which were geographically isolated and which had experienced different salinities over long periods of time, had varying capacities for the accumulation of proline. For example, *Armeria maritima*, the thrift, is a species which occurs both as a maritime halophyte and as a species of arctic-alpine habitats on many British mountains. Palaeoecological studies suggest that the species was widespread in distribution during the cold and disturbed conditions at the close of the last glaciation, some 10,000 years ago. Populations have subsequently been isolated on mountain tops and coastal habitats as forest has spread and eliminated this shade-intolerant species from many habitats. When tested under a variety of salinities, *Armeria* populations from inland situations showed only a limited capacity for proline accumulation. The response of coastal *Armeria* samples was about three times as great as those from inland. Such a difference in response is reminiscent of the varietal adaptation to proline accumulation as a response to drought found in barley (Singh *et al.*, 1972).

It is known that the enzymes of some halophytes are not able to operate in media of high salt concentration (for example, the work of Flowers, *J exp Bot*, **23**, 310, 1972, on *Sueda maritima*). Sodium chloride within halophytes must, therefore, be physically separated from the cellular enzyme systems, presumably by storage in the vacuole. Stewart and Lee have examined the effect of elevated proline levels upon the activity of several enzymes in *Triglochin maritima*, they found no depression of activity. They suggest, therefore, that proline could provide the means of cytoplasmic osmotic

adjustment in those halophytes where proline accumulation has been observed. Perhaps it serves a similar function in drought-stressed tissues where osmotic compensation may be required within the cytoplasm of affected cells. Apart from the elucidation of the precise role of proline in these processes, one of the most interesting developments in this work is likely to emerge from the exceptions to the general rules—exceptions like *Plantago maritima*.

Laser studies of atomic and molecular collisions

from G Duxbury

In the last fifteen years there has been an increasing interest in the experimental and theoretical study of the energy transfer processes occurring in atomic and molecular collisions. One of the main experimental techniques which has been used is that of crossed molecular beams, which requires very expensive ultra-high vacuum apparatus. An alternative way of obtaining the information is based upon experiments using single frequency narrow linewidth lasers. In these experiments use is made of the velocity selective interaction of monochromatic radiation with gaseous atoms and molecules. The information obtained from the laser experiments is similar to that from the crossed beam experiments, but does not require ultra-high vacuum techniques for its success. In addition, information can be gained, not only on the changes of translational energy, but also on changes in rotational energy. As examples of the types of experiment that can be carried out, two different ones are described, one of which has been carried out and one which has recently been proposed.

If a single-frequency laser emits radiation of frequency ν which differs slightly from the resonance frequency ν_0 of the atoms or molecules which absorb the radiation, the Doppler shift will make the laser radiation appear exactly on resonance only for those molecules whose component of velocity along the laser beam is $v = c(\nu - \nu_0)/\nu_0$, where c is the velocity of light. If the molecule irradiated is allowed to collide with a heavy target, the velocity dependence of the collision cross section for the production of a particular excited state can be determined by measuring the ratio of the fluorescence from this state, to that of the laser excited resonance fluorescence as the laser frequency is changed. Phillips and Pritchard (*Phys Rev Lett*, **33**, 1254, 1974) have proposed that this technique should give velocity resolutions comparable with that obtained

using the crossed molecular beam method, providing that the target is at least five to ten times heavier than the atom or molecule excited. They also suggest that since the velocity distribution in the laser experiment is known exactly, the true cross section can be deconvoluted from the measured one very accurately, so that this compensates for the slightly poorer velocity resolution of the laser experiment. It is proposed that, in some circumstances, state selection can be achieved by the use of a polarised laser beam. This should give a preferential orientation of either the orbital angular momentum vector, or of an electric dipole moment, along the direction of propagation of the radiation. The experiments described above are for the linear absorption region.

An alternative method of studying energy transfer that has already been demonstrated utilises saturation effects produced in low pressure gases by high power lasers. Under these conditions 'holes' are produced in the population of energy levels associated with the absorbing transition. Good examples of this type of behaviour are provided by the elegant double resonance experiments carried out by Freund, Johns, McKellar and Oka (*J chem Phys*, **59**, 3445, 1973). They studied two, three and four level systems in the vibration rotation spectrum of ammonia. The main effects that they demonstrated were the monitoring of a hole in the population of a vibration-rotation energy level caused by a strong two-photon pumping source, by a weak two-photon signal source, and the observation that holes burnt in the population of one level could be transferred to other levels by collisions. Two-photon pumping is not an essential requisite, and can be replaced by tunable laser sources once these become available. By fine tuning both the pumping and the monitoring sources different velocity molecules were studied, and by larger variations of the monitoring source energy transfer to different rotational energy levels was studied. Thus from one type of experiment it is possible to obtain information about both the magnitude of the intermolecular interaction $V(r)$ from the translational changes, and the angular dependence of the intermolecular interaction $V(\theta, \phi)$ from the rotational changes.

These sorts of study are only possible when the predominant cause of spectral line broadening is the Doppler effect, as in the infrared and visible regions. In the microwave region the lines are predominantly pressure broadened and double resonance then gives information only on rotational state changes, but not on changes in translational energy.

review article

Recent advances in electrical recording from the human brain

D. Regan*

New techniques for recording evoked potentials to sensory stimulation have led to applications with both theoretical and practical implications. Evoked potentials can be used as a diagnostic tool in some pathological conditions, as well as shedding light on the mechanisms by which sensory information is processed in the brain.

A FLOW of visual information to the eyes, auditory information to the ears or tactile information to the skin is represented by nervous discharges whose information content is successively reorganised as the signals pass from neurone to neurone on their way to visual, auditory or somatosensory areas of brain cortex where yet further processing takes place. The weak electrical currents generated here pass through the skull and can be recorded in the form of voltages, known as evoked potentials (EPs), between electrodes held on to the scalp. Although evoked potentials are generated mainly in the cortex, they can be used as tools to study the ways in which the brain sequentially processes sensory information as it ascends from peripheral to successively more central sites through the nervous system. They can also be used to reveal parallel processing in the brain, in which different types of information are handled simultaneously in separate channels.

During the past decade, descriptions of the ways in which single neurones in animal brains respond when the sensory end organ is stimulated have become a major influence in originating models of sensory information processing, and in this way have prompted a great many psychophysical studies designed to test the extrapolation of single-neurone studies to human perception. It is a long way from the electrical activities of nerve cells in cat or monkey brain to human conscious perception, and EP recording offers a promising means of bridging this interspecies gap. A second, quite different strength of EP recording is that it offers access to certain activities of both the normal and diseased brain that do not intrude into consciousness and so cannot be directly studied by psychophysics or subjective sensory tests. A third area in which EPs offer unique possibilities is in the objective testing of sensory function, especially in young children.

It is rare for a single sensory stimulus (for example one clap of the hands) to produce an EP so large and free from noise that it can clearly be seen in a normally amplified recording from scalp electrodes. More commonly the electrical activity of the rest of the brain is so prominent that the EP is buried in background noise. The most widely used method of enhancing the signal-to-noise ratio is signal averaging^{1,2}. An abrupt stimulus (for example a click) is repeated many (n) times, separated by intervals that are sufficiently long to allow the brain to settle down to its

undisturbed state between repetitions, and the n responses are electronically summed. If the EP to each stimulus is identical and occurs at exactly the same time after stimulation, the summed EP will be n times larger than a single EP, but the background noise will sum more slowly since it has no fixed relationship to the stimulus. This is called a transient EP because it is the transient response to an abrupt stimulus such as, for example, a brief flash of light, the

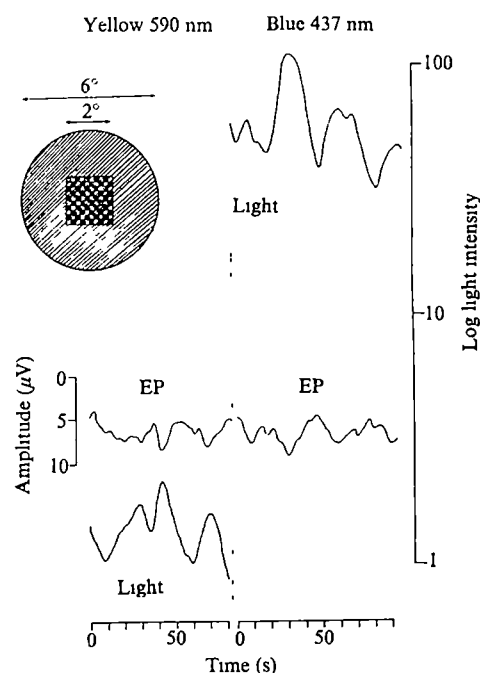


Fig 1 Evoked potential feedback when brain signals control the stimulus. The visual stimulus shown in the insert was a red pattern of bright and dark checks that exchanged places six times per second. Superposed on the pattern was a desensitising patch of light. The pattern elicited a 6-Hz evoked potential, whose amplitude continuously controlled the intensity of the desensitising light by means of a neutral density wedge in the desensitising beam. This feedback was set to maintain evoked potential amplitude constant at 6 μ V. When the colour of the desensitising light was changed from yellow to blue, the evoked potential immediately moved the wedge so as to increase the intensity of the desensitising light by about 40 times. Since the effect of the red (676 nm) pattern is restricted almost entirely to the red channel, this means that this channel is about 40 times more sensitive to yellow than to blue light. (Modified from Regan⁸)

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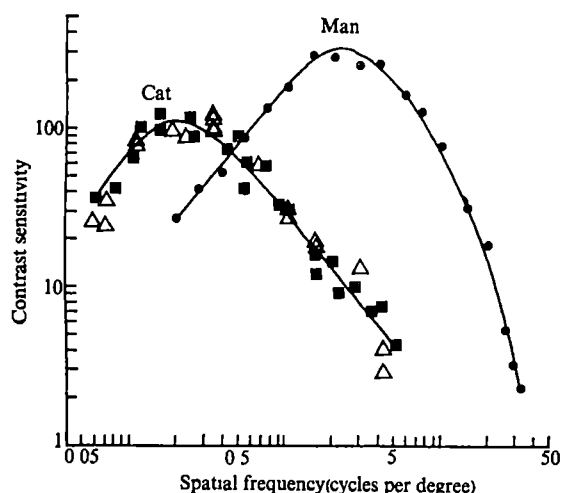


Fig. 2 Pattern vision in cat and man compared by means of evoked potential recording. The filled squares are the "contrast thresholds" determined by evoked potential recording for three cats¹⁶. The open triangles are contrast sensitivities determined behaviourally for two other cats¹⁷, and closely agrees with the evoked potential data. The solid circles are contrast sensitivities measured in man¹⁶, where evoked potential and psychophysical estimates agree also. In comparison with the human curve, the cat's is displaced to lower spatial frequencies by a factor of about ten, a somewhat similar effect would result from viewing the world through reversed binoculars so that everything looked ten times smaller.

onset of steady illumination, or the cessation of a long train of rapidly-repeated clicks.

Steady-state EPs are quite different from transient EPs. When a rapidly repeated stimulus is switched on, there is first a transient response which then gradually settles down to a steady-state EP waveform that repeats at the same frequency as the stimulus (f Hz)³⁻⁵. Here, the stimulus is precisely defined in frequency rather than time so that the steady-state EP can be recognised in the midst of the brain's background noise since it is composed of components (harmonics) whose frequencies are exactly f , $2f$, $3f$ Hz and so on. A Fourier analyser can automatically carry out the required computations, display the amplitude and latency of the steady-state EP, and confirm that the EP fulfils the necessary condition of remaining constant from moment to moment throughout the recording^{3,4}.

In some studies transient and steady-state EPs give complementary insights into brain function. From a practical point of view the steady-state method has the advantage of speed (it is up to 100 times faster)⁷, which may be especially important in clinical studies.

Using Fourier analysis of the waveform, EP amplitude can be displayed as a moment-to-moment running average, thus providing feedback to the experimenter so that he knows at once what result he has produced by changing some stimulus parameter^{3,6}. A different way of utilising this feedback is to arrange that, by electronic means, the EP itself continuously controls the stimulus so as to maintain EP amplitude constant⁸⁻¹⁰. Then the experimental subject's experiences are slightly disturbing if he consciously tries to blur a stimulus pattern by defocusing his eye, the stimulator opposes him so that the pattern's visibility does not seem to change. The experimenter, however, obtains through this technique a direct measurement of EP sensitivity, instead of an indirect one through amplitude measurements, with the advantages of increased precision and much shorter experiments as, for example, in measuring approximations to the action spectra of the eye's colour mechanisms⁸⁻¹¹ (Fig. 1).

Experimenters searching for the neural basis of conscious perception have looked for relationships between psychophysical measurements of sensation and evoked potentials

in human subjects. In such experiments, evoked potential recording offers a bridge between the detailed subjective reports and quantitative measures of conscious perception that can be obtained from human subjects and the recordings from individual nerve cells in the brains and sensory pathways of animals.

For example, the logarithmic relationship that has been shown to obtain between the amplitude of EPs elicited by a sine-wave grating and the objective contrast of the grating^{12,13} corresponds to the logarithmic relationship between stimulus contrast and subjective perception of contrast^{14,15}. The relationship between contrast and EP amplitude has been extended to cats^{16,17} and followed up with studies on the effect of grating contrast on the firing frequencies of single neurones on the cat's visual pathway¹⁸. These experiments showed that the logarithmic relationship held for just one class of neurones, the so-called simple cells of the visual cortex, whose behaviour thus seems to provide a basis for stimulus contrast perception.

The question of whether EP generation fails when the stimulus is made just too weak to produce a sensation has attracted a good deal of attention. There have been encouraging early reports of the use of EPs to auditory stimulation in assessing the bearing of babies and infants¹⁹⁻²³, although some later reports have been less optimistic²⁴. A straightforward way to estimate EP threshold is to graph EP amplitude against stimulus intensity in coordinates that give a straight line plot, and then extrapolate to zero EP amplitude. This method has been used for auditory EPs¹⁹⁻²³, and for visual pattern EPs^{12,13,25,28,29} in both cases giving EP thresholds that agreed well with subjective (psychophysical) thresholds. This latter finding for pattern EPs complements earlier reports that EPs could not be evoked by stimulating an eye with pattern during periods when pattern vision through that eye was suppressed by interocular rivalry³⁰ or by interocular suppression³¹⁻³³.

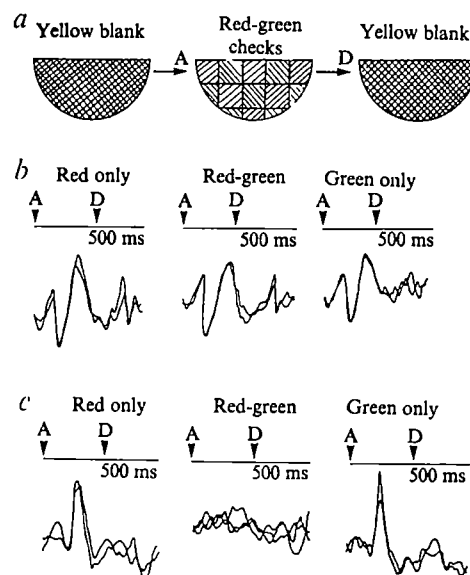


Fig. 3 Evoked potentials produced by a pattern defined only by colour differences. *a*, Stimulus: an unpatterned yellow patch abruptly changed to pattern of equiluminant red and yellow checks (appearance) and then back again (disappearance) without any change in total light flux. The traces marked red-green show that EPs to appearance (A) and disappearance (D) of pattern are clear in normal (*b*) but absent in colour blind (deuteranopic) subjects (*c*). The pattern of red-green checks is made up of a pattern of bright and dark red checks superposed on a pattern of bright and dark green checks. Traces marked red and green show that the constituent monochromatic patterns give clear EPs in both normal and colour blind subjects. (Modified from Regan and Spekreijse⁵²).

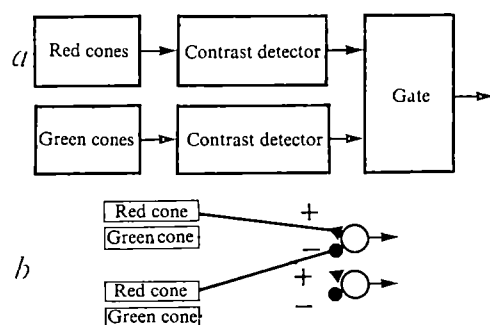


Fig 4 Pattern information is handled in parallel red and green channels which fuse before the stage of perception *a*, Red and green images cannot be simultaneously in sharp focus because of the eye's chromatic aberration, but when one or other image is momentarily sharp the corresponding colour channel feeds a strong signal to the gate, the function of which is to transmit preferentially the stronger pattern signal, possibly by means of inhibition between red and green pattern channels. Consequently it is the more sharply focused image that is seen at any given moment independently of whether it is the red or the green image, and furthermore beyond the gate the spectral sensitivity of the pattern channel is effectively photopic rather than being most sensitive to red or to green. *b*, Illustration of one possible arrangement of neuronal connections at the most peripheral stage. Excitatory signals shown by +, inhibitory by -. Uniform illumination by any colour gives zero final output. An output is, however, produced when a bright-dark boundary (or a red-green boundary) is positioned between the cone pairs. Note that this arrangement is by way of illustration only. Many other possible schemes of lateral inhibitory connection would also give sensitivity to spatial contrast borders.

Particularly for pattern stimulation, these findings offer an objective means of estimating psychophysical threshold in animals that may well prove important in future studies of the neural basis of sensation (Fig. 2). For example, such findings promise an alternative to the laborious behavioural methods in animals for detecting the effects on the visual pathway of an impoverished visual environment or artificial rotation of the visual world in early life.

It should not, however, be assumed that EP and psychophysical thresholds always coincide, even roughly. For example, flickering the intensity of an unpatterned patch of light gives EPs that bear no relation to subjective threshold. Whole experiments can easily be carried out with stimuli so weak that the subject never sees them³⁴. More significantly, the same increase of flicker frequency can increase EP amplitude, while abolishing the sensation of flicker^{3, 35}. This dramatic difference between EPs elicited by changing stimulus contrast (pattern) and by changing overall stimulus intensity is consistent with the suggestion that the two types of EP are generated by different populations of cortical cells³⁶.

In practice the two types of EP can easily be confused, since stimulating the eye with a pattern does not necessarily give a pattern EP. Although a fine pattern may give pure pattern EPs, a coarser version of the same pattern can give patterns responses mixed with EP components more or less similar to those elicited by flickering an unpatterned field^{29, 37, 38}. A curious consequence is that blurring a coarse pattern can increase the amplitude of pattern reversal though for fine patterns, as one would expect, blurring drastically attenuates the EP^{39, 40}. More recently it has been suggested that the second types of EP component produced by pattern reversal reflect the activities of movement detectors rather than luminance responses^{26, 41, 42}.

The original evidence for pattern detectors tuned to narrow ranges of spatial frequency in the human visual system was psychophysical⁴³⁻⁴⁵, now supported by evidence from EPs to sinewave grating stimuli which are sensitive to grating orientation and spatial frequency¹². Suggestions

based on psychophysical experiments that there exists a type of "feature filter", sensitive to an edge at some particular location in the visual field⁴⁶⁻⁴⁸ have been linked with the discovery of neurones in cat and monkey brains that are preferentially sensitive to light-dark boundaries in the visual field⁴⁹. Naturally, a sinewave grating covers a considerable area and also looks blurred at all times and would therefore not be expected to give EPs that emphasised the activities of local edge detectors. A pattern containing sharp edges, however, does elicit EPs that suggest some contribution from edge detectors. For example, EPs to a chequerboard pattern are almost abolished by occluding the edges of checks with very thin wires³⁷, and even very slight blurring markedly attenuates chequerboard EPs^{29, 32, 33, 40}, a result that, because of EP saturation, would not be expected if the effect of blur were caused entirely by contrast reduction⁵⁰.

Colour coding of pattern vision

A pattern that is defined only in terms of colour with no luminance differences can give clear EPs. For example a fine pattern of alternate equiluminant red and green checks or stripes elicits EPs when the red and green elements are interchanged^{27, 51} or when the pattern appears from a previously uniform yellow field⁵² (Fig. 3). Such EPs are markedly attenuated if the sharp edges of the bars or checks are even slightly blurred³³. These EPs are still large in the deliberately artificial situation when the eye's chromatic aberration is cancelled in such a way that both red and green images are sharply focused on to the retina⁵³. I have suggested that the existence of such EPs show that the physiological signals generated by red and green lights must still be segregated when they arrive at the most peripheral pattern-sensitive neurones in the eye (or brain). In other words, the most peripheral stage of pattern processing is

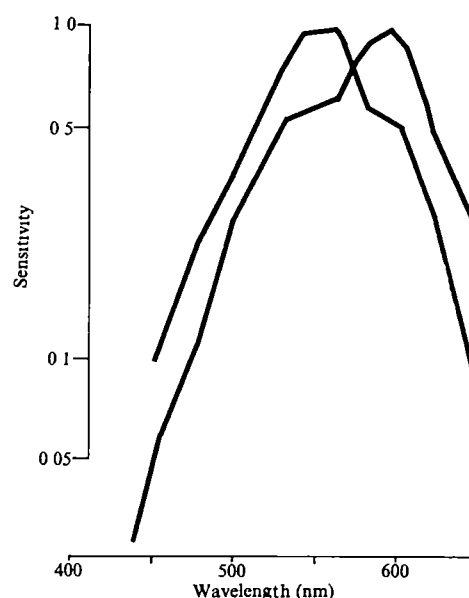


Fig 5 Spectral sensitivities for the red and green channels of pattern vision. The red channel (predominantly) was stimulated by a foveal red (676 nm) pattern of fine checks as in Fig. 1, and a desensitising patch of light was superposed on the red pattern. The intensities of the desensitising patch needed for a given attenuation of the red pattern EP were plotted (reciprocally) as ordinates against desensitising wavelength, giving the right hand curve. The experiment was repeated with a green (544 nm) pattern to obtain an approximation to the spectral sensitivity of the green channel (left hand curve). These spectral sensitivities approximate those of Stiles π mechanisms. Note because of the overlap of red and green spectral sensitivities, the colour channels are not completely separated by this technique (Modified from Regan⁹).

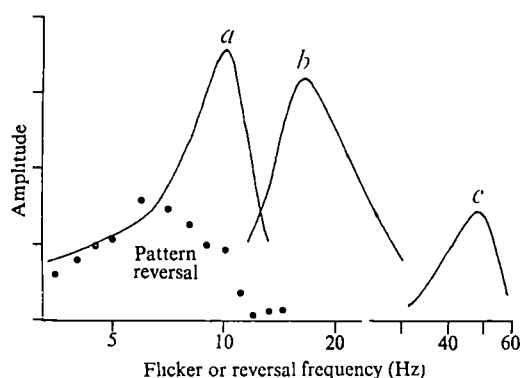


Fig. 6 *a*, Low, *b*, medium, and *c*, high frequency responses to flicker. The amplitudes of EPs produced by flickering an unpatterned patch of light show resonant-like peaks in three frequency regions. The properties of EPs are fairly uniform within a single frequency region, but are quite different in different frequency regions. EPs in the different frequency regions are generated in different areas of the brain, have different latencies, different colour properties and, furthermore, demyelinating disease slows medium frequency but not high frequency responses. The three types of signal may be separate from an early stage in the visual system. Note that these EPs to unpatterned flicker have quite different properties from EPs to fine patterns (whose frequency dependence is shown by dots) (Modified from Regan⁸⁶).

duplicated in parallel red and green-sensitive channels (Fig. 4). There is evidence that segregation is total⁵². In effect these channels treat a fine pattern defined in terms of different colours as though it were defined by differences in light intensity across borders. Thus, neural responses to fine patterns (for example checks smaller than about 20 arc min) can be explained without invoking opponent colour mechanisms.

In the case of fine patterns, an interesting effect occurs when the relative intensities of red and green checks are critically adjusted. The sharp borders between the checks become indistinct^{54,56}, particularly when the eye's chromatic aberrations are cancelled⁵³. Significantly, red-green edges are minimally distinct when red and green luminances are equal⁵⁴⁻⁵⁶. Nevertheless, EPs to fine patterns are large at the balance point and show no sign of a minimum^{26,27,53}. This suggests that the red and green pattern channels, although separate at the most peripheral stage, fuse before the stage of perception in such a way that the resulting single pattern channel has the eye's photopic spectral sensitivity (Fig. 4).

Here the charm of EP recording is that a neural organisation is clearly revealed even though its operation is not subjectively evident and does not intrude into perception.

One function of this peripheral colour segregation might be to minimise the degradation of visual acuity caused by the eye's chromatic aberration. Red and green lights from a pattern are never simultaneously in sharp focus on the retina, so that a pattern detector sensitive to a wide range of colours would always receive a blurred retinal image. If, however, the most peripheral stage were segregated into two colour channels, then at any moment either the red or the green channel would receive a more sharply-focused image and preferentially feed into the next stage (Fig. 4). This would explain why visual acuity has been reported to be little worse in white than in monochromatic light^{57,60}, why acuity for one colour can be unaffected by superposing light of a different colour^{61-64,66}, why the eye's depth of focus is greater in white than in monochromatic light⁶⁶, and would also be in accord with the McCollough effect⁶⁵.

This is not the whole story, however. There seem to be additional detectors in the human brain that take advantage of colour differences in such a way that we see spatial patterns more clearly⁵³. These detectors respond preferentially to borders between differently coloured areas. Unlike

the intensity-pattern detectors described above, they are sensitive to chromatic contrast *per se* and may involve opponent colour mechanisms. Their activity is more evident for coarse than fine patterns¹²³.

Evoked potentials to a pattern of equiluminant red and green checks are a sensitive test for colour blindness⁵², and can give some insight into the condition. For example in one colour blind (deuteranopic) subject, EP amplitude could be completely abolished by carefully balancing the intensities of red and green checks⁵² (Fig. 3), which suggests that ocular chromatic aberration was almost ineffective⁵³ and therefore only one colour channel is present. Indeed in a later study¹¹, measurements of the pattern channel's action spectrum showed that the deuteranope investigated had a red mechanism only.

Approximations to the action spectra for the red and green channels of human pattern vision, measured objectively by recording EPs elicited by a pattern of fine checks directed on to the fovea⁸⁻¹¹, are similar to the action spectra of red and green retinal photopigments or the red and green colour mechanisms of Stiles (Fig. 5). In these experiments EP recording has the advantage over psychophysical methods that approximations to action spectra can be straightforwardly measured, not only near the threshold of pattern perception, but also at high, everyday levels of pattern sensation and with very little added adapting light. The point here is that if intense adapting lights are used, the effect of either red or green cones will be selectively reduced by chromatic adaptation so that separate colour mechanisms will be observed even if red and green signals are not segregated at the contrast stage. Only if very little adapting light is used can it be confirmed that red and green signals really are segregated at all times.

The blue channel of pattern vision is not evident when the eye is stimulated with a blue pattern, even when the

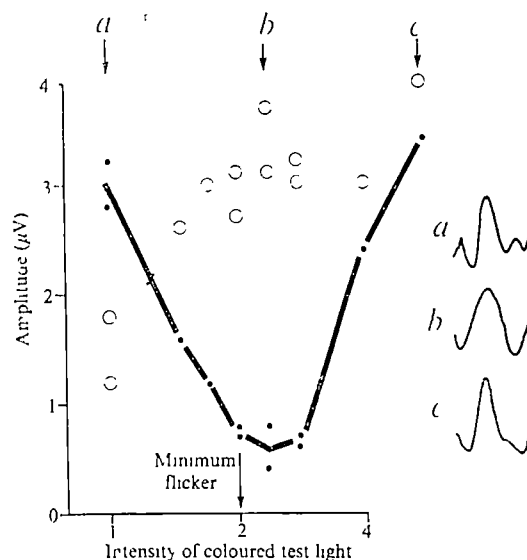


Fig. 7 A patch of standard white light was alternated with a patch of blue light 24 times/s. The blue light's intensity was adjusted by the subject until he saw minimum flicker (arrowed, 2 on abscissa). At this point, therefore, the blue test light and standard white light had the same photometric luminance. The steady-state EP was composed of two frequency components: —, a medium-frequency component of 24 Hz and — — —, a high-frequency component of 48 Hz. The high-frequency EP component fell to a minimum close to the point of minimum subjective flicker (continuous line), so that its amplitude correlated with the photometric luminance of the blue light. In contrast, the medium-frequency EP component (broken line) showed no minimum and was large even when the light did not appear to flicker at all (at arrow). This component therefore did not correlate with photometric luminance. Other evidence shows that it correlates with stimulus colour. Note that the averaged waveform (*a*, *b* and *c*) arbitrarily confounded the two components and was virtually uninterpretable (Modified from Regan⁸⁴).

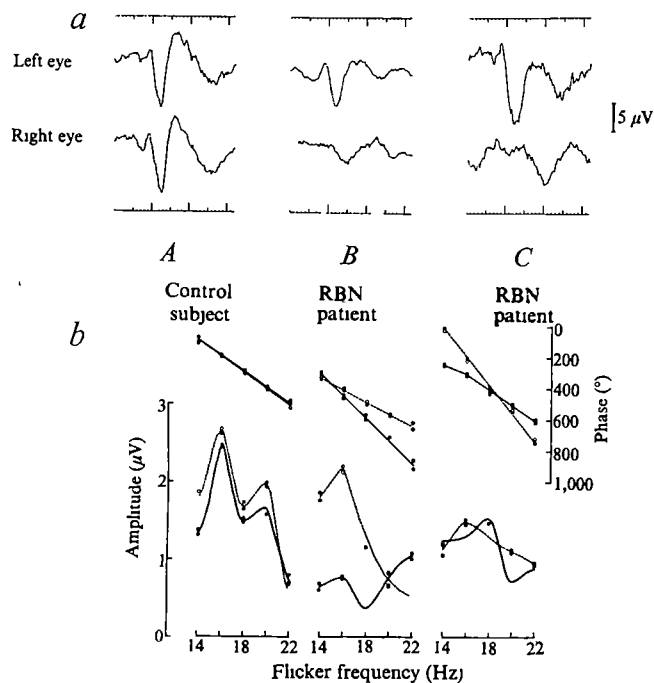


Fig 8 Delayed visual evoked potential in patient with multiple sclerosis *a*, Transient evoked potentials to pattern for a healthy subject (*A*) and two patients (*B*, *C*) who were recovering from acute attacks of retrobulbar neuritis in the right eye. Note the delayed and attenuated responses from the affected eyes. Time (abscissae) divided into 100, 50 and 10 ms (From Halliday *et al*⁸⁹) *b*, Steady-state evoked potentials elicited by flickering an unpatterned patch of light for a healthy control subject and for two retrobulbar neuritis (RBN) patients —, The right eye, — —, the left eye. Note that for the affected eyes the plots of evoked potential phase against flicker frequency are steeper than for the good eyes (that is, the evoked potential is delayed for the affected eyes). This can be seen to hold independently of whether amplitude is attenuated (From Milner *et al*⁸⁷)

checks are as coarse as 40', presumably because the blue mechanism is insensitive. It can be revealed by using intense yellow chromatic adaptation in the same way that Stiles isolated his blue π mechanisms (ref 67 and D R, unpublished observations). More precise measurements on colour blind subjects have recently shown, that the blue pattern channel's action spectrum approximates that of Stiles' blue π mechanism, even in the absence of chromatic adaptation¹¹.

The colour properties of pattern EPs described above are quite different from the colour properties of EPs elicited by unpatterned stimuli as described in the next section.

Unpatterned coloured stimuli

The aim of revealing differences between the ways in which the eye handles light of different colours has led several experimenters to compare EPs elicited by red, green, blue and yellow flashes. In experiments where different colours were reported to give different EP waveforms the stimulus fields seem to have been so large that they illuminated an inhomogeneous retinal region including rods as well as cones⁶⁸⁻⁷¹. In one crucial experiment stimulation was restricted to the fovea, a 2° central retinal area that is relatively homogeneous and mediates good colour vision⁷². This experiment did not support the notion that different colour sensations are associated with different EP waveforms, since red, yellow, green and blue flashes now gave transient EPs the waveforms of which were similar.

On the basis of EP latency rather than waveform Krauskopf was able to distinguish the separate contributions of red, green and (the slowest) blue chromatic mechanisms even when he used small (1°) foveal stimuli⁷³. Adapting Stiles' increment technique to EP work, Krauskopf used chromatic adaptation to select one or other chromatic

mechanism and obtained EP evidence that red, green and blue activities, separate at the most peripheral level, do not converge until at least 50 ms after stimulation. It has often been suggested that differences between the time courses of neural signals elicited by red, green and blue light may be the brain's physical representation of hue⁷⁴⁻⁷⁷. This notion might, perhaps, be open to test in man by further EP applications of Stiles' techniques for isolating colour mechanisms.

Although there seems to have been surprisingly little success in identifying EP correlates of the colour sensations aroused by monochromatic lights, the accompanying luminosity sensations seem to have clear EP correlates. Armington found that the amplitudes of some components of transient EPs to flash correlate with photometric luminance*, in other words they have the same spectral sensitivity as the light-adapted eye^{78,79}. Flickering an unpatterned patch of light gives steady-state EPs that are often composed of two frequency components, one at the flicker frequency and one at twice the flicker frequency. The two frequency components have quite different colour properties, provided that the frequency of one ("medium frequency") component falls in the range 13-25 Hz while that of the other ("high frequency") component fall in the range 35-60 Hz (Fig 6). The amplitude of the high frequency component can be used to measure the spectral sensitivity curve of the eye with rapidity and a precision quite equal to that of the conventional psychophysical method (better than 0.05 log unit)^{34,81}. This method has already been used in goldfish⁸² and promises a simple alternative to behavioural methods in the monkey.

Although this high frequency component of the EP therefore correlates with photometric luminance, the medium frequency component does not, even though it is part of the same averaged waveform (Fig 7). Medium frequency components do not even have a unique spectral efficiency curve⁸³, are very sensitive to chromatic adaptation^{81,84,85} and may reflect the activities of opponent colour mechanisms⁸⁶. The latencies of medium and high frequency EPs differ by about 2:1 (roughly 120 and 60 ms respectively)^{5,34,81,84,88}. High frequency and medium frequency EP components, carrying different visual information might well be anatomically separate, travelling along parallel channels, very early in the visual system (see below).

Clinical applications of evoked potentials

Two factors that initially prompted clinical application of EP recording were (1) the need for objective tests of sensory function in young children and babies where conventional ways of testing vision and hearing are uncertain, and (2) the failure of some EPs to correlate with sensation, which means that they offer access to some activities of sensory pathways not accessible to subjective tests. Both these possibilities have recently become practicalities, and it seems that indeed there are certain specific clinical diagnostic problems where custom-designed EP methods can usefully supplement conventional sensory tests.

One example is that of multiple sclerosis, in which breakdown of the insulating myelin sheath surrounding the axons (conducting fibres) of nerves eventually leads to impaired or blocked nervous conduction. This demyelination characteristically occurs at several sites in the central nervous system and multiple sclerosis can be difficult to diagnose when there is evidence of demyelination at only one site. This problem can be eased by using recently developed EP tests that detect damage to the visual pathway caused by retrobulbar neuritis, a common precursor of multiple sclerosis.

* "Photometric luminance" is not quite the same as "brightness". Even when intensity (and therefore photometric luminance) is held constant, the brightness of patterned stimulus can be altered by manipulating the patterning⁸⁰. In this manoeuvre, EP amplitude correlates with photometric luminance rather than brightness⁴⁰.

- Transient EPs to pattern stimulation are significantly delayed in almost all patients after such an attack⁸⁹⁻⁹¹, steady-state EPs to pattern are also delayed^{87,88}. Flashing an unpatterned field, however, gives transient EPs that have been reported to provide no reliable indication of retrobulbar neuritis, though there is some disagreement on this point⁸⁹⁻⁹⁴. On the other hand flickering an unpatterned field elicits steady-state EPs that are reliably delayed in all patients for flicker frequencies in the medium-frequency region between 13 and 25 cs^{-1} (Figs 6 and 8)^{87,88}. The flicker test for retrobulbar neuritis has the advantage over the pattern test that it can be used even when the patient's visual acuity is low. Curiously, flicker EPs were delayed for no patients when flicker frequencies lay in the high-frequency region between 35 and 60 cs^{-1} ^{87,88}. This difference is an example of the ability of EPs to provide information not accessible to sensory tests, since neither medium-frequency nor high frequency EP amplitude correlates with flicker perception^{3,6,26,35}. The difference might be explained if the two types of flicker signal travel along different classes of axons in the optic nerve, one of which is preferentially demyelinated.

In a few patients delays may reach 100–180 ms⁸⁷⁻⁹¹. Such large delays are difficult to explain entirely in terms of slowed conduction along myelinated nerve fibres^{87,88}.

In patients in whom, on clinical evidence, demyelination seems restricted to the spinal cord, a high percentage have delayed transient EPs to pattern, and this evidence of visual pathology has proved useful in diagnosing multiple sclerosis^{89-91,95}. Interestingly, when steady-state EPs are recorded the flicker and pattern tests may pick up different patients, suggesting that the two tests are sensitive to different aspects of visual damage caused by multiple sclerosis⁹⁵.

Refraction and amblyopia

Spectacles can be prescribed by recording evoked potentials to pattern, since the largest responses are produced when the pattern looks sharpest^{7,96-101}. As little as one minute's recording is required when the power of the test lens is varied continuously during the examination⁷.

The clinician faces a more difficult problem when his patient cannot see fine detail, but spectacle lenses fail to improve vision. In this condition (amblyopia) the visual defect seems to lie in the networks of nerve cells in the retina and brain that handle pattern information. It seems likely that a period in early life during which a distorted image of the world is formed on the retina can be a major cause of this abnormal neural development. Childhood squint, and uncorrected long sightedness in one eye only are common causes of amblyopia¹⁰²⁻¹⁰⁴, uncorrected astigmatism can lastingly impair the ability to see straight lines of one particular orientation ("meridional amblyopia")^{106,107}. If allowed to persist until the child is too old, amblyopia becomes irreversible and the child may be left with only one good eye. During early childhood amblyopia can be cured by simple treatments such as by occluding the good eye for a period, normal vision then gradually returns to the amblyopic eye¹⁰²⁻¹⁰⁴. Accurate estimates of the patient's visual acuity are clearly important for effective treatment. Unfortunately it is during early childhood, the time when treatment is most effective, that reliable estimates of visual acuity are most difficult to obtain, especially in realistic conditions where several different retinal regions are simultaneously stimulated^{102,104}. Recording EPs to patterned stimuli offers a means not only of studying the mechanism of amblyopia but also of estimating the visual acuity of young children, and thus of improving treatment of amblyopia^{108,113} including meridional amblyopia^{7,114,115}.

There is disagreement whether stimulating the amblyopic eye's fovea gives normal EPs^{107,109-112}, but it does seem clear that the retinal area immediately surrounding the fovea (perifovea) give larger EPs for the amblyopic than

for the good eye¹¹⁰⁻¹¹². When fovea and perifovea are stimulated together (as in everyday life) foveal responses are relatively depressed¹¹⁰⁻¹¹² suggesting that different retinal regions interact abnormally. EPs from amblyopic and good eye are inverted in polarity, and this difference disappears after successful treatment¹¹².

If EP recording is to become a practical way of testing vision in young children it must be developed into a much briefer test. A speedy method developed with this in mind is to project on to a screen a checkerboard pattern whose dark and bright squares exchange places six times per second while slowly zooming up and down in size once every 30 s. The 6-Hz EP to the pattern is continuously plotted against check size, and several such graphs are electronically averaged. The child's attention is maintained during the 2–3 min required by projecting a cartoon film on to the checked pattern¹¹³.

Where binocular vision seems normal it is now possible to test stereoscopic depth vision objectively, since EPs can be recorded that are specific to changes in binocular disparity (that is, to movements in depth)^{116,117}.

Abnormal visual projections in albinos

In many animals including man the projection from each eye is divided so that signals from the right half of each retina go to the right hemisphere of the brain, and from the left hemiretina to the left hemisphere. Guillery and others have found that, in the albinos of several species, signals from a patch of retina near the midline are erroneously routed to the opposite hemisphere, so breaking up the orderly left-to-right projection of the retinal image on to the cortex¹¹⁸⁻¹²². Guillery has suggested that the squint commonly found in these albinos may be a functionally useful adaptation to this misrouting.

Evoked potential recording offers one means of testing whether visual signals from part of the retina are routed to the wrong side of the brain in human albinos. When an albino subject viewed a visual stimulus with one eye only, the resulting EP seemed to be generated in one rather than both hemispheres^{123,124}. Although it is difficult to carry out visual EP tests on albinos with nystagmus, these results do indicate that EP recording offers a means of finding whether any patient with defective vision might have an underlying abnormality in the routing of his retino-cortical connections.

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articles

Surface reactivity of tungsten

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Pre-adsorbed nitrogen or β -carbon monoxide inhibit the surface reactivity of tungsten towards hydrogen, both in catalysis and in adsorption. The effect of nitrogen can be satisfactorily accounted for by a simple reduction in the area of reactive filament, taking into account the selective adsorption of this gas by the low index planes of tungsten. With carbon monoxide the effect is considerably greater, perhaps because of lateral interactions between adsorbed hydrogen and carbon monoxide.

ISOTOPE equilibration reactions taking place on the surface of a metal are among the simplest examples of heterogeneous

catalysis and thus provide a suitable starting point for investigating in detail the catalytic process. One particularly attractive feature of the study of these reactions is the excellence of the agreement observed between experiments carried out on polycrystalline wires of quite different origin¹⁻⁶. In addition to the intrinsic interest of such studies, it is possible to use the equilibration reaction as a chemical probe to investigate the surface. Thus, when a tungsten wire was used as a catalyst for nitrogen, carbon monoxide or hydrogen isotope equilibration^{4,6,7} it was found that poisoning by pre-adsorbed oxygen was nearly linearly dependent on the fractional oxygen coverage. From this it was inferred that active patches⁸ did not form a significant part of the surface. Furthermore, combination of the results of these experiments with measurements of the adsorptive characteristics of the surface (the saturation uptake and the initial

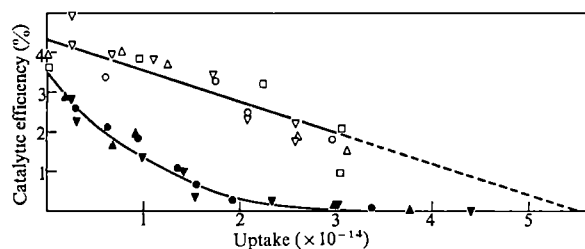


Fig 1 Inhibition of hydrogen isotope equilibration at room temperature by adsorbed nitrogen (\circ , ∇ , Δ , \square) and by adsorbed carbon monoxide (\blacktriangle , \bullet , \blacktriangledown). Total pressure of hydrogen isotopes ($\times 10^8$) \square , 5.6, ∇ , 25, \circ , 28, Δ , 61, \blacktriangle , 5.1, \bullet , 24, \blacktriangledown , 26. Pumping constant for HD $0.48 \pm 0.07 \text{ s}^{-1}$. Uptakes measured as atoms cm^{-2} for nitrogen and molecules cm^{-2} for carbon monoxide.

sticking probability) shed light on the close, yet elusive, relationship between the centres of catalysis and chemisorption. Some further evidence on this point has come from studies of the catalytic activity of the low index planes of tungsten [(100), (110), and (111)] in ammonia decomposition⁹, in which it was reported that the activities of the planes were different, as are their reactivities towards nitrogen¹⁰.

Because nitrogen and carbon monoxide are both more strongly bonded in their β states to polycrystalline tungsten than is hydrogen, these gases might be expected to inhibit the activity of tungsten as a hydrogen isotope equilibration catalyst. If this were to happen it would then be possible to compare the effects of a dissociated-molecule inhibitor¹⁰ (N_2) with the probably, though not certainly, undissociated carbon monoxide¹⁰⁻¹². It is this comparison we report here.

Measuring catalytic activity

The same apparatus was used as for the earlier experiments⁴. It consists of a Pyrex glass ultra-high vacuum (UHV) chamber ($p_{\text{min}} < 5 \times 10^{-10}$ torr) in which the experimental filament (length 20.7 cm and geometric area 0.83 cm^2) is suspended. The filament had been cleaned previously by extensive heating in oxygen and its reactivity was monitored regularly during the present series of experiments, by observing the uptake and initial sticking probability of nitrogen. The constancy of these quantities during the course of the work was taken as sufficient evidence that the filament did not become contaminated. Flashing to about 2,040 K *in vacuo* sufficed to remove adsorbed gases and was the normal method of cleaning.

The experimental gases were admitted through all-metal valves from either of two gas storage volumes. Adjustment of the apertures of these valves produced the required partial pressure of a gas. A similar valve connecting the UHV chamber

to the pump controlled the rate of flow of a gas over the filament. Partial pressures were measured with an omegatron radio-frequency mass spectrometer which formed part of the UHV section. A commercial Bayard-Alpert ionisation gauge (Edwards High Vacuum) with a low temperature, lanthanum hexaboride-coated rhenium filament was used to calibrate the mass spectrometer. The catalytic activity of the filament was measured by streaming a mixture of approximately equal partial pressures of hydrogen and deuterium over it and observing the formation of HD at the expense of H_2 and D_2 .

The efficiency E of the filament as an equilibration catalyst has been defined previously⁷ by the ratio

$$E = \frac{\text{Observed rate of production of HD}}{\text{Maximum possible rate of production of HD}}$$

The way in which the heights of the mass spectrometer peaks, recorded under steady state conditions, are used to calculate E

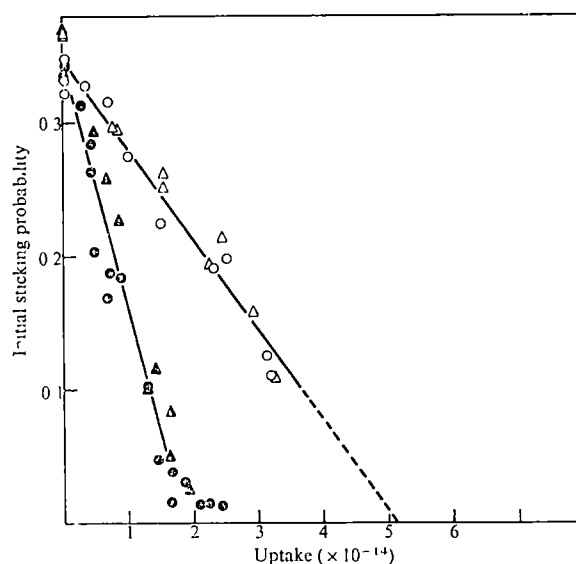
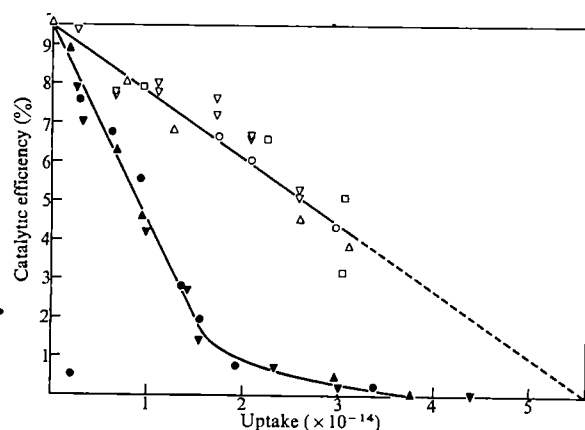


Fig 3 Effect of pre-adsorbed nitrogen or carbon monoxide on s_0 of hydrogen (\circ or \bullet) or deuterium (Δ or \blacktriangle). Uptakes of nitrogen or carbon monoxide as in Fig 1.

Fig 2 Inhibition of hydrogen isotope equilibration at elevated temperatures by adsorbed nitrogen (800 K) and by adsorbed carbon monoxide (690 K). Symbols as in Fig 1.



has been fully described elsewhere⁷. Earlier experiments have shown that E is independent of pressure within the range 2×10^{-7} – 9×10^{-7} torr (ref. 7), and a total pressure between 5×10^{-8} and 6×10^{-7} torr was used in these experiments. The catalytic inhibiting effect of pre-adsorbed nitrogen or carbon monoxide was investigated as follows. The filament was cleaned and cooled to room temperature *in vacuo*. Nitrogen or carbon monoxide was then let in and the filament was allowed to adsorb for the appropriate time. After cutting off the gas supply the filament was heated briefly to 650 K to equilibrate the adsorbed layer and to remove any gas in the weaker α state¹¹. The hydrogen–deuterium mixture was then streamed over the filament at a chosen temperature and when the peak heights of masses 2, 3 and 4 were constant they were recorded. Contamination of the filament by the background gas (largely CO) presented more of a problem in this work than it had done previously, because it was not possible to flash off impurities accumulated from the background without disturbing the initial layer. This difficulty was particularly marked with CO which is reversibly adsorbed by the walls of the UHV chamber^{13,14}. The experimental time for measurements of catalytic activity at any particular coverage was thus limited to about 0.5 h. After the measurements had been made the uptake of carbon monoxide was determined by heating the filament in stages, to 650 K to remove the hydrogen and, when the gas had pumped away, to 1,610 K to desorb carbon

monoxide. A record of the pressure burst caused by this desorbed gas was made with a rapid-response amplifier and ultraviolet recorder, from which record the uptake was calculated. Nitrogen uptakes were calculated from the partial pressure record, made with the omegatron, during the initial adsorption¹⁵.

The effects of pre-adsorbed nitrogen or carbon monoxide on the adsorptive characteristics of the filament, as defined by the initial sticking probability s_0 and the maximum uptake θ_{H_2} at a pressure near 1×10^{-7} torr, were determined in a separate series of experiments. To do this the filament was partially covered as before and hydrogen or deuterium was admitted at a constant rate from a storage volume. The partial pressure during the adsorption period was recorded with the omegatron until the filament was saturated. s_0 and θ_{H_2} were both calculated from this record¹⁵.

The effects of the two gases on the efficiency at room temperature are shown in Fig. 1 and in the plateau region⁷ (690 K for CO, 800 K for N_2) in Fig. 2. Within a set of experiments carried out under fixed conditions the relative values of E are consistent, though the absolute accuracy is not better than $\pm 25\%$ (ref. 7). The ways in which s_0 and θ_{H_2} depend on the uptakes of CO or N_2 are shown in Figs 3 and 4.

Implications

The discussion of these results and their relationship to the earlier observations of the effect of pre-adsorbed oxygen atoms on the surface reactivity of this filament towards nitrogen, hydrogen and carbon monoxide gives an opportunity of reviewing the conclusions that can be drawn from experiments of this type about the chemical constitution of the surface. In so doing it is appropriate to relate the work, where possible, to the conclusions drawn from other techniques. But, in view of the proliferation of papers relating to the surface of tungsten the comparison must be selective.

We shall take as the starting point for discussion a consideration of the relevant geometric factors. Tungsten has the b.c.c. structure and it has been suggested that a well annealed polycrystalline filament will expose predominantly the (100) and (110) planes^{16,17}. As far as the influence of nitrogen on the catalytic efficiency is concerned, this suggestion provides an immediate interpretation of the considerable remaining catalytic activity of the nitrogen-saturated filament, roughly half that of the clean filament, because nitrogen adsorption on tungsten shows a strong surface-plane dependence. A careful review of the evidence reached the conclusion that dissociative chemisorption occurs on W(100) at room temperature, but that the W(110) plane is unreactive¹⁰. Subsequent experiments have either confirmed this conclusion^{18,19} or have demonstrated that, at most, adsorption takes place with a sticking probability which is initially very low (0.005) and which thereafter declines rapidly²⁰. On the other hand hydrogen shows no such geometric sensitivity and adsorbs readily and extensively on a polycrystalline tungsten filament^{7,16,21,22} and on both of these single crystal planes²³⁻²⁶. The catalytic activity towards hydrogen of the nitrogen-free surface can thus be attributed to both (100) and (110) planes. Saturation with nitrogen then removes the reactive sites on (100) planes but leaves the (110) planes clean and still reactive.

The near-linear decline in efficiency with increasing nitrogen uptake is closely paralleled by the fall in s_0 and θ_{H_2} . In this, the effect of nitrogen closely resembles the way in which the first stage of oxygen adsorption reduces the activity of this filament towards hydrogen (though oxygen will eventually eliminate all reactivity) and the results can be interpreted (as for O-atom adsorption) as the consequence of a reduction in the area of reactive surface directly proportional to the uptake of the pre-adsorbed gas. The similarity between oxygen and nitrogen extends further, in that for both gases the reactivity is halved by a coverage of about 2.7×10^{14} atoms cm^{-2} . But, whereas for nitrogen all the atoms are on the (100) plane, the non-selective adsorption of oxygen by tungsten²⁷⁻²⁹ presumably leads to a

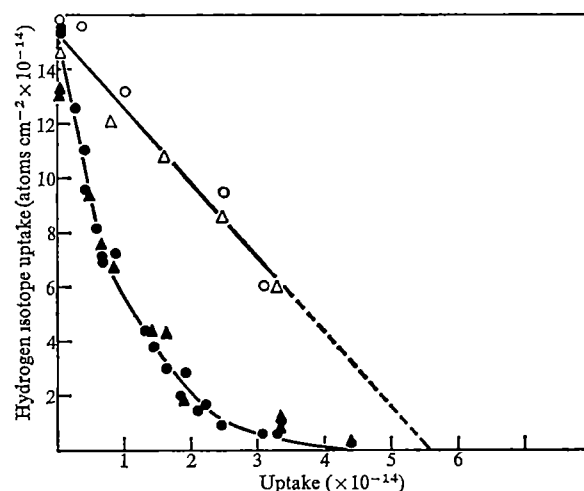


Fig. 4 Effect of pre-adsorbed nitrogen or carbon monoxide on maximum room-temperature uptake of hydrogen (O or ●) or deuterium (Δ or ▲). Uptakes of nitrogen or carbon monoxide as in Fig. 1.

distribution of oxygen atoms over both the (100) and (110) planes. It is interesting to note that this chemical similarity between adsorbed oxygen and nitrogen is reflected in the formation of ordered geometric arrangements, as detected by LEED (low energy electron diffraction), of the atoms of both gases on the (100) plane^{30,31}.

There are two previous experiments concerned with the co-adsorption of nitrogen and hydrogen on tungsten which are of particular relevance to this work^{21,32}. These experiments were principally concerned with the uptakes resulting from sequential adsorption of nitrogen and hydrogen and with the ability of nitrogen to replace previously adsorbed hydrogen on either a polycrystalline wire²¹ or a W(100) single crystal plane³². The major conclusion drawn from both of the earlier experiments, and one with which the results presented here are in complete accord, was that there is very little interaction between adsorbed hydrogen and adsorbed nitrogen.

We now turn to a consideration of the effect of pre-adsorbed carbon monoxide on the reactivity of tungsten. The method used for preparing a surface partially covered with CO ensured that the gas was held in the more strongly bonded of the surface states of CO on tungsten, the β states, the α -CO having been desorbed by the prior heating to 650 K (ref. 33). The β state is itself composite and consists of three substates, normally called β_1 , β_2 and β_3 in ascending order of binding strength. As expected these were poorly resolved under the conditions of our experiments¹⁴ though with special care separation is possible³³. In adsorption, the β_2 and β_3 states fill up first so that the early part of the catalytic inhibition curve is associated with the poisoning effect of carbon monoxide in these states. As Figs 1 and 2 show $(\beta_2 + \beta_3)$ -CO has a profound effect on the reactivity of tungsten, an uptake which is only about 40% of β -CO saturation reduces E by about 90%. This uptake has a comparable effect on s_0 and θ_{max} as Figs 3 and 4 show. It is thus clear that a CO molecule is much more effective at deactivating the surface than either an oxygen atom or a nitrogen atom. This observation again agrees very satisfactorily with the earlier measurements of the co-adsorption of carbon monoxide and hydrogen^{21,34}. The more recent of these studies showed that adsorption of CO weakened the bonding of hydrogen to the surface (in this case the (100) plane) thereby generating a new state which was desorbed at 273 K but which was retained on the surface at lower temperatures. A fractional coverage of CO equal to 0.4 was sufficient to exclude all β -hydrogen from the surface at 273 K. Bearing in mind that this observation relates to a monocrystalline surface and the results in Figs 1-4 are for a polycrystalline surface, the concordance between the two sets of experiments is striking. It has been pointed out, however,

that the characteristic desorption spectrum of β -CO from tungsten can be accounted for satisfactorily starting from the postulate that carbon monoxide adsorption takes place dissociatively and that lateral, repulsive interactions are present between the adsorbed atoms³⁵

The initial purpose of this series of experiments was an attempt to locate the sites of catalytic activity on a tungsten surface. There seems to be little doubt that for the reactions chosen to probe the surface, which probably come into the category of 'facile'³⁶, much of the surface which is capable of adsorbing the gases is also catalytically active. It will be interesting to discover whether this is also true of more complex reactions.

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The nucleotide sequence of an RNA polymerase binding site on bacteriophage fd DNA

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The nucleotide sequence of the RNA polymerase binding site at a promoter on fd RF DNA has been determined in comparison with the starting sequence of RNA initiated at this promoter. The RNA polymerase binding site contained the startpoint of transcription in the centre and a region with twofold symmetry in the non-transcribed part.

SEVERAL species of RNA with unique starting sequences and sizes are transcribed on the doubly closed replicative form (RF-I) DNA of fd bacteriophage¹. This suggests that fd RF-I DNA provides sets of specific sites for initiation and termination of transcription. To analyse the structure at such an RNA initiation site (promoter), we first constructed the cleavage map of RF-I DNA using *Haemophilus* restriction endonucleases, and then localised the promoters on the map. We found that a short DNA segment, Hap-Hga V, contained one of the promoters for G-start RNA (Fig. 1), the fragment representing about 3.8% of the length of fd DNA and corresponding to about 230 base pairs. A uniform RNA of about 120 bases long was efficiently synthesised on this fragment. The DNA fragment was specifically bound to RNA polymerase with GTP present. As the binding of the polymerase to DNA renders the covered portion of the DNA resistant to DNase^{2,3}, the region protected by the polymerase was isolated from Hap-Hga V. We compared the nucleotide sequence of the polymerase binding site isolated in this way with the sequence of RNA initiated on Hap-Hga V, based on the transcription of the DNA sequence into RNA in the oligonucleotide-primed reaction⁴.

Preparation of fd RF-I DNA, *E. coli* RNA polymerase, and restriction endonucleases (*Hap* and *Hga*) from *H. aphrophilus* and *H. gallinarum* was as previously described^{3,5}. For preparation of Hap-Hga V, a complete digest of RF-I DNA with *Hap* and *Hga* was prepared⁵, mixed with RNA polymerase and GTP, DNA fragments which formed stable complexes with the

polymerase were isolated as in the legend to Fig. 2b. The two fragments (Hap C and Hap-Hga V in Fig. 2b) isolated by this

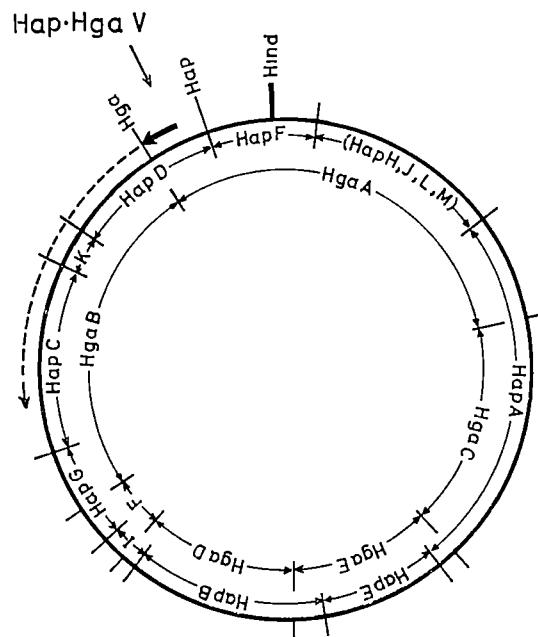


Fig. 1 The location of Hap-Hga V on the cleavage map of fd DNA. The map has been constructed using the cleavages with *Hap*, *Hga* and *Hind* (a restriction endonuclease from *H. influenzae* Rd). Hap A,B — and Hga A,B ---- label of fragments created by *Hap* and *Hga* (our unpublished observations). Hap-Hga V is produced either by digestion of Hap D with *Hga* or by digestion of Hga A with *Hap*. The G-start RNA initiated on Hap-Hga V proceeds in the counter-clockwise direction and terminates at a site on Hap C.

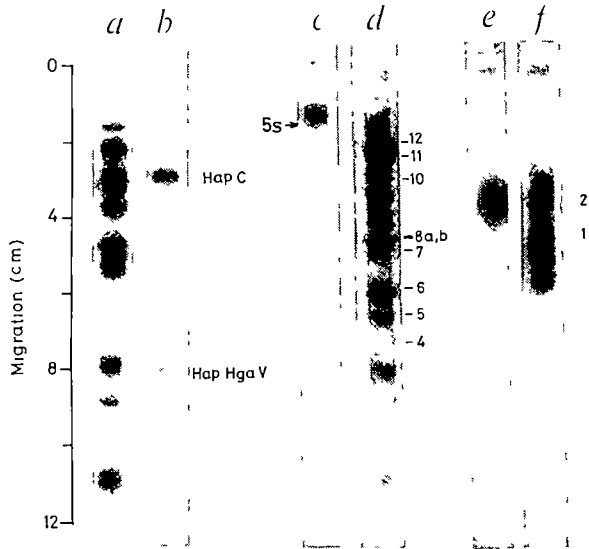


Fig 2 Gel electrophoretic patterns of (a) a digest of fd RF-I DNA with *Hap* and *Hga*, (b) DNA fragments which bind to RNA polymerase with GTP present, (c) RNA synthesised on Hap-Hga V, (d) a partial T₁ RNase digest of RNA made on Hap-Hga V, (e) the RNA polymerase binding site isolated from Hap-Hga V, and (f) (A)₃ U-primed RNA formed on the polymerase binding site. (a) A complete digest of ³²P-RF-I DNA with *Hap* and *Hga* was layered on a 5% polyacrylamide gel column (0.6 cm × 12 cm), electrophoresed for 12 h at 2 mA per tube, and the autoradiograph was taken as in ref 5. (b) The Hap-Hga digest of ³²P-RF-I DNA (0.1 mg) was added to a binding mixture (3 ml) containing 8 mM MgCl₂, 50 mM KCl, 40 mM Tris (pH 7.9), 0.1 mM GTP, and about 100 pmol RNA polymerase. After 20 min at 30°C, the mixture was passed through a Millipore filter (HA 0.45 μm). The filter was washed with 1 M KCl, 8 mM MgCl₂, 20 mM Tris (pH 7.9) and then with 8 mM MgCl₂, 20 mM Tris (pH 7.9). DNA fragments retained on the filter were eluted with 0.2% SDS–20 mM Tris (pH 7.9). The eluate was deproteinised, concentrated and electrophoresed as in (a). (c) Hap-Hga V (non-labelled, about 2 pmol) was added to a reaction mixture (1 ml) containing 8 mM MgCl₂, 50 mM KCl, 40 mM Tris (pH 7.9), 0.1 mM dithiothreitol, 0.1 mM α-³²P-GTP (10⁷ c.p.m./nmol), 0.2 mM each of three other nucleotide triphosphates, and about 10 pmol RNA polymerase. Incubation for 30 min at 37°C was terminated by shaking with 80% phenol. The aqueous layer was passed through a Sephadex G50 column (1 cm × 30 cm). The RNA fraction was collected, and electrophoresed on a 15% gel column for 3 h at 150 V as in ref 7. Under these conditions, about 7 pmol of RNA were synthesised. Arrow shows position of 5S ribosomal RNA as marker. (d) ³²P-RNA synthesised on Hap-Hga V was partially digested with T₁ RNase as in ref 6, and the digest was electrophoresed as in (c). The numbered bands were extracted, rerun on 15% gels containing 7 M urea, and submitted to sequence analysis. (e) Hap-Hga V (labelled with ³²P, about 20 pmol) was added to the binding mixture given in (b). After 10 min at 30°C, 50 μg of DNase I were added and incubation was continued for an additional 30 min. Digestion was terminated by shaking with 80% phenol. The aqueous layer was treated with ethylether, dialysed against 0.1 mM EDTA–20 mM Tris (pH 7.9), and electrophoresed on a 15% gel as in (c). (f) The portion of Hap-Hga V protected from DNase I digestion (about 4 pmol) was heated for 5 min at 100°C, rapidly cooled, and added to a reaction mixture (1 ml) containing 8 mM MgCl₂, 50 mM KCl, 40 mM Tris (pH 7.9), 0.1 mM dithiothreitol, 10 μM α-³²P-GTP (5 × 10⁷ c.p.m./nmol), and 20 μM each of three other nucleotide triphosphates, 10 μM AAAU, and about 10 pmol of RNA polymerase. Incubation was carried out for 3 h at 37°C, and terminated by shaking with 80% phenol. After removal of phenol with ethylether, the template was digested with DNase I. The solution was deproteinised, and passed through a Sephadex G50 column. The RNA fraction collected was mixed with 50 μg of fd SS DNA. NaCl was added to 0.9 M and sodium citrate to 0.09 M. The mixture was heated for 5 min at 100°C, held for 3 h at 51°C, slowly cooled, and passed through a Millipore filter. The filter was washed with 0.3 M NaCl, 0.03 M sodium citrate. RNA retained on the filter was dissociated by heating for 5 min at 100°C in 20 mM Tris (pH 7.9), and electrophoresed on a 15% gel column containing 7 M urea, as in (c).

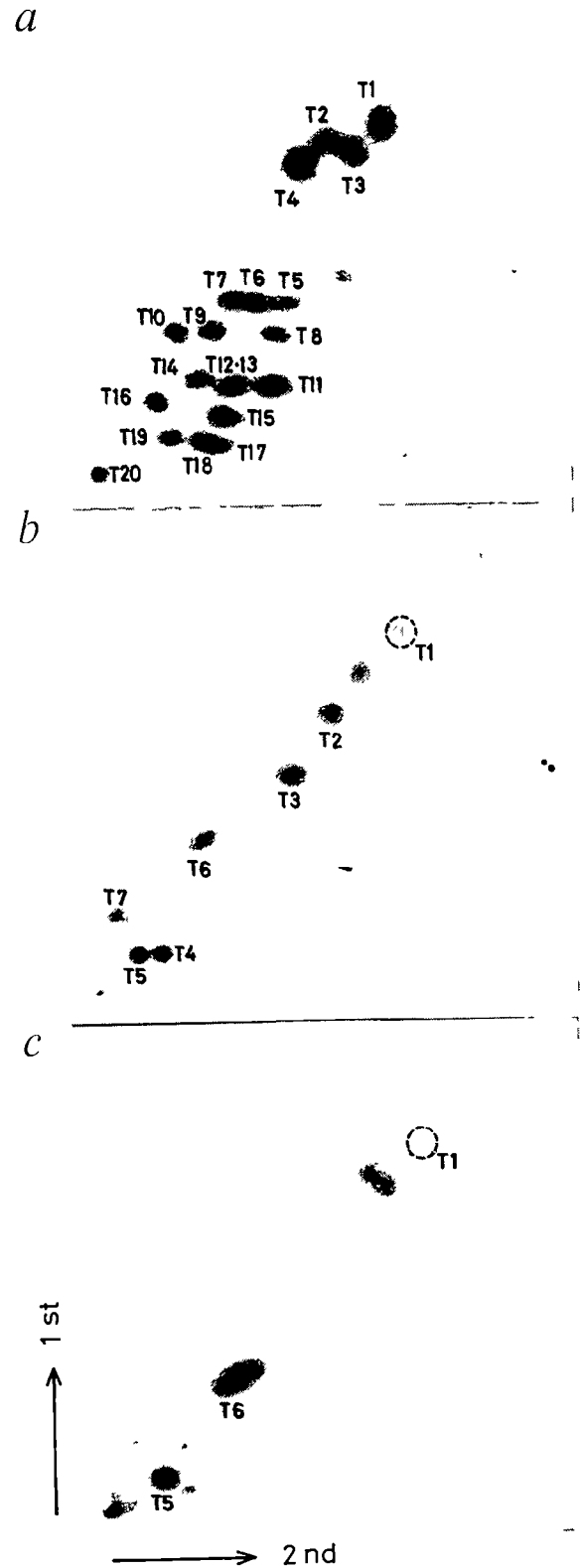


Fig 3 Two-dimensional fingerprints of T₁ RNase digests of (a) RNA synthesised on Hap-Hga V, and (b), (c) (A)₃ U-primed RNA formed on the RNA polymerase binding site. (a) (*G) RNA was synthesised on Hap-Hga V as in the legend to Fig 2c and digested with T₁ RNase. The digest was applied to a 20 cm × 20 cm PEI-cellulose plate and chromatographed as in ref 6. The sequences of the spots numbered are given in Table 1. (b) and (c) (*G) RNA was synthesised on the heat-denatured RNA polymerase binding site and fractionated as in the legend to Fig 2f. Bands 1 and 2 indicated in Fig 2f were digested with T₁ RNase and chromatographed as in (a). (b) Fingerprint of Band 2. (c) Fingerprint of Band 1. The sequences of spots numbered are given in Table 2.

Table 1 Analysis of nucleotides in pancreatic and T₁ RNase digests of RNA formed on Hap-Hga V

Spot no	T ₁ RNase products Sequence*	Molar ratio	Spot no	Pancreatic RNase products Sequence*	Molar ratio
T1a	G <u>A</u>	2	P1a	C <u>A</u>	3
T1b	G <u>G</u>	2	P1b	C <u>C</u>	2
T1c	G <u>U</u>	1	P1c	C <u>G</u>	2
T2	CG <u>G</u>	1	P1d	C <u>U</u>	4
T3	AG <u>G</u>	1	P2a	GC <u>A</u>	1
T4a	CAG <u>U</u>	1	P2b	GC <u>G</u>	1
T4b	ACG <u>C</u>	1	P2c	GC <u>U</u>	1
T4c	ACG <u>A</u>	1	P3	GAC <u>G</u>	1
T5	UAAAG <u>A</u>	1	P4	GAAC <u>U</u>	1
T6	AACUG <u>U</u>	1	P5	AAAGC <u>A</u>	1
T7	ACCUG <u>A</u>	1	P6	AAAGAC <u>C</u>	1
T8	UCUG <u>C</u>	1	P7	GGAC <u>G</u>	1
T9	AUUCG <u>C</u>	1	P8a	U <u>A</u>	4
T10	CUAUCAG <u>U</u>	1	P8b	U <u>C</u>	5
T11	UAUUG <u>G</u>	1	P8c	U <u>G</u>	7
T12	CAUUUG <u>A</u>	1	P8d	U <u>U</u>	9
T13	UUUAAAG <u>C</u>	1	P9a	AU <u>C</u>	1
T14	AUUCAAUG <u>A</u>	1	P9b	AU <u>G</u>	2
T15	AUUUAUG <u>G</u>	1	P9c	AU <u>U</u>	4
T16	UCAUUCUG <u>U</u>	1	P10	AAU <u>G</u>	1
T17	AUUUUUG <u>A</u>	1	P11	GU <u>U</u>	2
T18	UUUUCUG <u>A</u>	1	P12a	GAU <u>U</u>	3
T19	AAUAUUUAUG <u>A</u>	1	P12b	AGU <u>A</u>	1
T20	pppG <u>U</u>	1	P12c	AGU <u>C</u>	1
			P13	GAAU <u>A</u>	1
			P14	GGU <u>C</u>	1
			P15	GAGGGGAU <u>U</u>	1
			P16	pppGU <u>A</u>	1
			P17	GGAAU <u>C</u>	0.5

* Nucleotide linked to the 5'-end is underlined

procedure were separated by sucrose density-gradient centrifugation. On adding Hap-Hga V to an RNA synthesising mixture, a uniform RNA which migrated near 5S ribosomal RNA as marker was efficiently synthesised (Fig 2c). The RNA only hybridised to the (–) strand of the template, like those made on intact RF-I DNA. For isolation of the RNA polymerase binding site, Hap-Hga V was mixed with RNA polymerase and GTP, digested with DNase I and the portion protected was isolated as in the legend to Fig 2e. The gel electrophoretic pattern of the polymerase binding site prepared by this procedure is shown in Fig 2e. The average chain length estimated from the analysis of the terminal P was roughly 45. To synthesise RNA on this fragment, the fragment was denatured by heating, and incubated in an RNA synthesising mixture containing a primer oligonucleotide. The synthesised RNA was isolated, and fractionated by hybridisation to fd single-stranded (SS) RNA [(+) strand]. The RNA fraction complementary to fd SS DNA was collected, and further resolved by gel electrophoresis (Fig 2f). The detailed conditions for RNA synthesis and the procedure for fractionation of RNA are given in the legend to Fig 2f. Hydrolysis of RNA with RNases and analysis

of the digests by two-dimensional chromatography on PEI (polyethylenimine)–cellulose plates were as described previously⁶

Sequence of RNA synthesised

RNA was synthesised on Hap-Hga V with each of the four nucleoside α³²P-triphosphates (abbreviated as (*N)RNA, where N = A, C, G, or U), and hydrolysed with either pancreatic RNase or T₁ RNase. The resulting nucleotides were resolved on PEI–cellulose plates. A typical fingerprint obtained from (*G)RNA is shown in Fig 3a. The distribution of ³²P in each spot was determined. In addition, the nearest neighbour analysis was carried out on each spot after the complete digestion with T₂ RNase. All the nucleotides obtained and their approximate molar ratios are summarised in Table 1.

The sequence of shorter oligonucleotides was determined simply by the distribution of ³²P in nucleotides. For analysis of longer oligonucleotides, each digestion product was further cleaved using either pancreatic RNase or T₁ RNase, and chromatographed. The products were digested with T₂ RNase, and ³²P in nucleotides determined. The sequences for all the

Table 2 Analysis of nucleotides in pancreatic and T₁ RNase digests of (A)₃ U-primed RNA formed on the RNA polymerase binding site

Spot no	T ₁ RNase products Sequence †	Molar ratio	Spot no	Pancreatic RNase products* Sequence †	Molar ratio
T1	G <u>U</u>	1	P1	C <u>C</u>	1
T2	AAG <u>C</u>	0.8	P2	AC <u>C</u>	1
T3	UCAG <u>A</u>	1	P3	AAAU <u>C</u>	1
T4	UCUAUUUAUAG <u>U</u>	1	P4	AAAAAU <u>C</u>	0.4
T5†	U(CUU)UACCCUG <u>U</u>	1	P5	GU <u>C</u>	1
T6	AAAUACAG <u>G</u>	0.6	P6	AGU <u>C</u>	1
T7	AAAUCAAAAUCAG <u>G</u>	0.4	P7	AGGU <u>C</u>	1
			P8	AGAAGC <u>A</u>	0.7

* The products obtained from (*C)RNA are listed

† Nucleotide linked to the 5'-end is underlined

‡ The position of C in parentheses is not determined by the nearest neighbour analysis

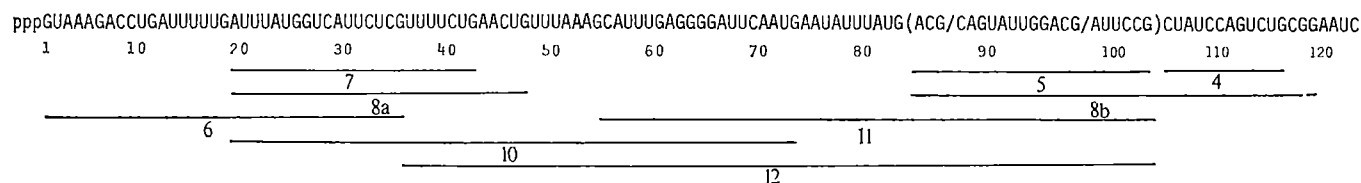


Fig 4 The nucleotide sequence of RNA synthesised on Hap-Hga V. The region covered by each partial T_1 RNase product is underlined. The numbers represent the bands indicated by the side of Fig 2d. Each band has been repurified using gel electrophoresis in urea.

oligonucleotides were determined in this way, except those for spots T10, T16, T18 and T19 in the T_1 RNase digest. The nearest-neighbour analysis of T10 deduced four possible sequences, CAUCCUAG, CCUAUCAG, CCAUCUAG, and CUAUCCAG. As the nucleotide linked to the 3'-end is G, we looked for three possible sequences, GCA, GCC and GCU, in the pancreatic RNase digest. Table 1 shows only one GCU in P2c and two GCA in P2a and P5. As two GCA appear in T4a and T12 in the T_1 RNase digest, we concluded that the sequence for T10 is CUAUCCAG. The analysis of T16 deduced two possible sequences, UCAUUCUCG and UCUCAUUCG. The sequence was determined to be UCAUUCUGG by digestion with U_2 RNase. Two alternative sequences were also obtained for T19, AAUAUUUAUG and AUUUAAUAUG. Two possible sequences, GAAUA and GAUU, were looked for in the pancreatic RNase digest. There are three GAUU in P12a and one GAAUA in P13. The three GAUU appear in T9, T14 and T15 in the T_1 RNase digest. Thus the sequence for T19 was deduced to be AAUAUUUAUG. The nearest-neighbour analysis did not provide information on the position of C in the U cluster of T18. This sequence has been determined to be UUUUCUG in a separate experiment, in which the sequence of RNA formed on the (+) strand of Hap-Hga V in the oligonucleotide primed reaction was analysed. The sequences of most oligonucleotides in Table 1 have also been confirmed by this type of analysis.

To reconstruct RNA from the T_1 RNase products, partial T_1 RNase digests were prepared and resolved by gel electrophoresis (Fig 2d). The resulting bands were redigested with

reduced RNA synthesis was stimulated approximately threefold by adding $(A)_3U$ as primer. We chose this oligonucleotide as primer because RNA formed on Hap-Hga V contained the sequences, AUUU and AUUUUU, near the starting end (see Fig 4).

RNA was synthesised with each of the four nucleoside $\alpha^{32}P$ -triphosphates, fractionated by hybridisation to fd SS DNA, and resolved by gel electrophoresis. Note that the direction of RNA thus prepared is the reverse of the RNA made on 'intact' Hap-Hga V. The major band obtained (Band 2 in Fig 2f) was digested with T_1 RNase and chromatographed on PEI-cellulose plates. A typical fingerprint obtained from $(^*G)RNA$ is shown in Fig 3b. The major spots detected are listed in Table 2. The amounts of spots T1 to T5 were about equimolar. The sequence of each spot was analysed as in the previous section. For analysis of longer oligonucleotides, digestion was carried out using either pancreatic RNase or U_2 RNase. Unique sequences were obtained for all spots, except for T5. The sequence deduced for T5 was U(CUU)UACCCUG, in which the position of C in parentheses was not determined. Spots T6 and T7 contained AAAU at the 5'-end and overlapped with the sequence at the 3'-end. These sequences are just complementary to the sequence which either positions 9 to 15 or positions 9 to 22 on the RNA made on Hap-Hga V (see Fig 4). In addition, the sum of T6 and T7 was about equimolar with each other spot (see Table 2). Thus, we concluded that the leftward RNA analysed had been initiated with primer at either position 15 or position 22 on the sequence in Fig 4. On the basis of analysis on nucleotides adjacent to the terminal G, all the

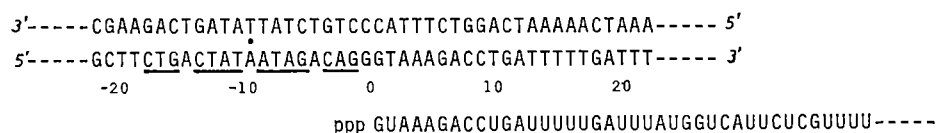


Fig 5 The nucleotide sequence of the RNA polymerase binding site on Hap-Hga V. The starting sequence of RNA formed on Hap-Hga V is indicated for comparison. The region with twofold symmetry is underlined. The axis of symmetry is at the -10 position.

T_1 RNase and chromatographed on PEI-cellulose plates. On the basis of the result obtained and information on the nucleotide adjacent to the terminal G of each spot, all the T_1 RNase products were ordered into a sequence of RNA, except those for the region between the positions 84 and 104 (Fig 4). The arrangement of three segments in parentheses is not determined yet. The yield of P17(GGAAU) which was assigned to the 3'-end of the RNA was less than one mole in the pancreatic RNase digest (see Table 1). This suggests that the RNA chains were randomly terminated after position 120.

Sequence of RNA polymerase binding site

As shown in Fig 2e, the fraction of Hap-Hga V which was protected from nucleolytic digestion by the polymerase binding gave a relatively uniform band on gel electrophoresis. To analyse the sequence of such a region, the DNA fragment isolated was denatured by heating and added to an RNA synthesising mixture in which the substrate concentration was

T_1 RNase products obtained can be ordered either (1) T6(T7) T1 T4 T5 T3 T2 or (2) T6(T7) T1 T5 T4 T3 T2. The combination was determined as (2) using the analysis of Band 1 in Fig 2f. Band 1 is assumed to be an intermediate, as this band was shifted to the Band 2 region by extending the incubation period. On chromatography of the T_1 RNase digest of Band 1, the major spots detected were only T1, T5 and T6. In Fig 3c, a fingerprint obtained from $(^*G)RNA$ is shown. Note that spot T1 is only obtained from $(^*U)RNA$. In other experiments, T7 was also detected in addition to these three spots. According to this combination, the sequence subsequent to T6(T7) becomes complementary to the starting sequence of RNA made on Hap-Hga V.

In Fig 5, the sequence of RNA finally deduced is shown as the sequence of DNA. The sequence has been confirmed by the analysis of the pancreatic RNase products (Table 2). The sequence would represent only the major part of the polymerase binding site. Nevertheless, the size of the sequence determined was about equal to the average size of the DNA fragment isolated as the polymerase binding site. Figure 5 shows that the

right half of the sequence is just identical with the starting sequence of RNA initiated on Hap-Hga V, indicating that transcription is initiated in the centre of the binding site. The other feature of the sequence is that a region with twofold symmetry is contained in the non-transcribed part, as indicated in Fig. 5. Such symmetrical regions have been detected in the polymerase binding site of other promoters^{8,9}, although each sequence is different. Initiation of transcription would include at least the processes of recognition of the promoter signal and formation of an initiation complex by the polymerase. If these processes occur at different parts of a promoter, the polymerase binding site we analysed would be a part of the promoter. As mentioned earlier, RNA chains are efficiently initiated on Hap-Hga V indicating that this fragment contains all of the sequence information needed for the promoter function. The length of the non-transcribed region of this fragment is about 100 base pairs. Sequencing of such a region will provide more information about the feature of the promoter.

In the course of this study, we learned that Schaller, Gray and

Herrmann¹⁰ have determined the sequence of a strong RNA polymerase binding site on fd by direct DNA sequencing. The sequence obtained by these authors coincides with the sequence of the polymerase binding site presented in this paper. They also note a region with strong symmetry in the non-transcribed part of the binding site.

We thank Dr Schaller for the exchange of unpublished information.

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letters to nature

Radiation as a source of gravitation

I HAVE investigated a few steady state cases according to general relativity, and some of the results are of interest.

Throughout this letter, relativistic units ($c = 1$, $G = 1$), and ray optics are used.

(1) Spherical symmetry, purely radial rays. The metric is

$$ds^2 = e^{2\nu} dt^2 - (1 - 2\Phi)^{-1} dr^2 - r^2(d\theta^2 + \sin^2\theta d\psi^2) \\ \nu = \nu(r), \quad \Phi = \Phi(r) \quad (1)$$

Imposing $T = T_2^2 = T_3^3 = 0$ and defining

$$4\pi r^2 T_0^0 = -4\pi r^2 T_1^1 = \varepsilon(\Phi)$$

the equations reduce to

$$\frac{d\varepsilon}{d\Phi} = \frac{2\varepsilon}{1-2\Phi} \frac{\Phi + \varepsilon}{\Phi - \varepsilon}, \quad \frac{1}{r} \frac{dr}{d\Phi} = -\frac{1}{\Phi - \varepsilon}, \quad \varepsilon e^{2\nu} = \text{constant} \quad (2)$$

Note that ε , a pure number, is the sum of the equal inward and outward fluxes of radiation, and that, for comparison, the

outward flux of the Sun is $\varepsilon = 10^{-26}$, while a quasar might perhaps reach 10^{-4} .

The solutions of equation (2), in the relevant region ($\Phi < \frac{1}{2}$, $\varepsilon \geq 0$) can be expressed in closed form in terms of $x = (\frac{1}{2} + \varepsilon)[\varepsilon(1 - 2\Phi)]^{-1/2}$. They start at the origin along $\varepsilon = \Phi$ (Fig. 1) (region A) then sharply slice off towards the right, proceed towards higher values of Φ with an only imperceptibly increasing ε (assumed to be fairly small) (region B) until, shortly before $\Phi = \frac{1}{2}$ is reached, ε begins to grow rapidly, with Φ soon passing a maximum and then diminishing (region C), while ε still grows. The line $\Phi = 0$ is crossed, and ε grows until $\varepsilon = -\Phi$ whence (region D) $\varepsilon \rightarrow 0$ as $\Phi \rightarrow -\infty$. Note that the origin corresponds to $r = \infty$, that the radius drops very sharply along the curve in region A, quite fast in B, but only gently as we proceed through C and D with $r \rightarrow 0$ as $\Phi \rightarrow -\infty$.

It is immediately plain that our normal picture of such a system is represented by A and the adjacent parts of B, and that C and D are the unexpectedly peculiar neighbourhood of the centre where focusing produces a curious singularity, probably irreproducible in practice owing to the non-zero wavelength of light.

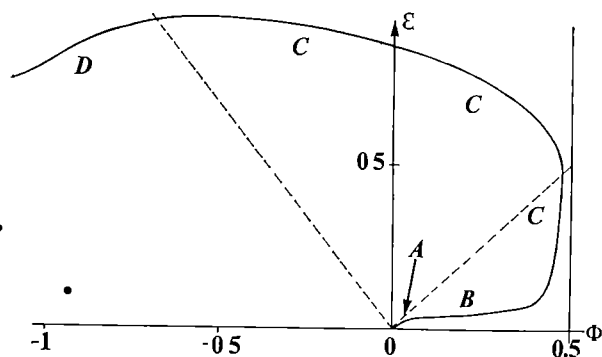
To avoid the centre, radiation may be limited to $r \geq r_1$ by putting a material body in. Most simply, if at $r = r_1$ there is a thin material shell with positive T and T_0^0 and empty interior, then necessarily

$$0 < \Phi_1 < 4/9, \quad \varepsilon_1 < \frac{1}{2}[1 - (1 - 2\Phi_1)^{1/2}][3(1 - 2\Phi_1)^{1/2} - 1] \quad (3)$$

so that we are confined to regions A and B. The zone of radiation may also be terminated by an outer shell, with empty space beyond. Though the conditions are a little more complex than at an inner shell, in fact the radiation pressure helps to support the shell.

Without an internal body, the central conditions do not select any particular solution of equation (2). Indeed the central singularity is such that on this ray picture it should be possible to confine, by an external shell, the zone of radiation to C and D, and even to terminate it with Φ so negative that, even with the necessarily positive mass of the shell, the external solution is one of negative mass.

Fig. 1 Schematic diagram of a typical solution of equation (2)



(2) As a second example, one could treat this case in a quasi-Newtonian fashion by taking the flux ϵ to be constant. Therefore the energy content and thus supposedly the mass would depend linearly on the radius, being proportional to it if the radiation extends to the centre. In this last case the potential Φ would equal ϵ and be constant throughout (corresponding to region *A* above), in the more general case of a central body of mass exceeding that of its volume filled with appropriate radiation $\Phi - \epsilon$ would be a positive multiple of r^{-1} (corresponding to region *B* above), whereas there seems to be no relativistic case corresponding to a central body with mass less than that of its volume filled with the continuation of the radiation.

Note that metric (1), with $\Phi = \text{constant}$, $rdv/dr = \text{constant}$, leads to a non-trivial constant energy tensor T_{μ}^{α} which, for small equal and opposite values of the constants, gives radial radiation in the first approximation.

(3) Cases of cylindrical symmetry. These may be static or stationary depending on whether the fluxes parallel to the positive and negative z directions are everywhere equal or not.

Consider now a static case characterised by an amplitude A and apply a Lorentz transformation of velocity V parallel to the z axis. The flux against V will be enhanced and that in the opposite direction diminished. Now let $V \rightarrow 1$, while letting $A \rightarrow 0$ suitably. In the limit one arrives at a system with a unidirectional flow of energy. Unidirectional cases so derived form a special class (which of course could have been derived directly) with the property that all rays are parallel to the axis. The existence of this class is somewhat surprising since after all the stream of energy near the axis should act as a source of gravitation leading to rays weaving in and out, crossing each other along the axis of symmetry and being pulled back from further out by the gravitational force, as is indeed the case in general for cylindrically symmetrical systems.

With the appropriate general metric (all coefficients are functions of r only)

$$ds^2 = e^{2\sigma} [e^{2\lambda} \cos 2\gamma dt^2 + 2 \sin 2\gamma dt dz - e^{-2\lambda} \cos 2\gamma dz^2] - e^{2\delta} dr^2 - r^2 d\psi^2 \quad (4)$$

this particular class is given by

$$\delta = \sigma = 0, \quad w(\gamma) \equiv r d\gamma/dr = \pm r \cos 2\gamma d\lambda/dr$$

giving

$$8\pi T^{00} = \frac{e^{-2\lambda}}{2r^2(\pm 1 - \sin 2\gamma)} \frac{d}{d\gamma} (w^2 \cos^2 2\gamma)$$

naturally with the trace of the energy tensor and its radial and transverse components vanishing.

(4) More generally, acceptable solutions of equation (4) representing pure radiation without transverse (ψ) components are described by equation (4) in the notation $u = r d\sigma/dr$, $v = r d\lambda/dr$, $w = r d\gamma/dr$ by

$$\delta = 2\sigma,$$

$$2rdu/dr = 2u + u^2 - v^2 \cos 2\gamma + w^2 \geq 0,$$

$$rdv/dr \cos 2\gamma \geq 4vw \sin 2\gamma,$$

$$(rdv/dr \cos 2\gamma - 4vw \sin 2\gamma)^2 \geq (rdu/dr)^2 + (rdw/dr - 2v^2 \sin 2\gamma \cos 2\gamma)^2$$

equality in the last case implying unidirectional flow of energy. Full details will be published elsewhere.

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Spectra of the Jupiter radio bursts

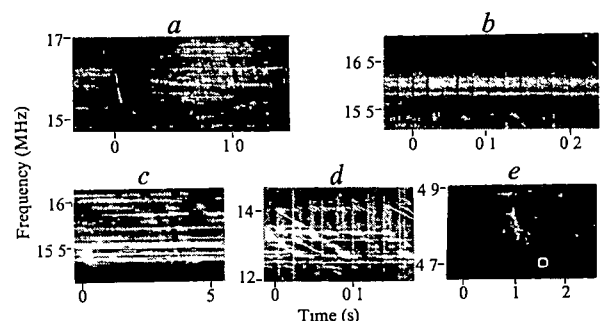
BETWEEN March and September of 1973 and 1974, observations were made of the Jupiter radio bursts with the Llanherne filled-aperture low frequency radio telescope¹ (650 m \times 650 m). The signals over the frequency range 4–29 MHz were recorded directly on to six video tape recorders for 5 min each day near transit. Some observations at 15–17 MHz were also obtained between May and September 1972. Frequency-time spectra were subsequently made with a 256-channel real-time analyser with channels 10 kHz wide and separated by 10 kHz. The spectra were recorded on 35-mm film moving at a variety of speeds from 150 inch min⁻¹ to 3 inch min⁻¹. When records with higher frequency resolution (2 kHz) or time resolution (100 μ s) were required, a time expansion spectrum analyser² with 1,500 channels in a 3 MHz bandwidth was used. Over the observing period, tape records of the Jupiter radio bursts were obtained during about two-thirds of all transits, and so far about 80 h of video tape containing the bursts has been accumulated. Here I present a preliminary description of the chief properties of their spectra.

Like the solar radio bursts, the dynamic spectra provide a convenient basis for classification, and the existence of at least two classes, the L bursts with a time scale of seconds and the S bursts with a time scale of milliseconds, has been recognised by earlier observers^{3,4}. But with the better time and frequency resolution now available it has become clear that there is considerably more variety in the radio phenomena as well as much more fine structure.

The L or lane bursts are seen on the frequency-time ($f-t$) plane as a diffuse background crossed by drifting modulation lanes (Fig. 1*a*). The ($f-t$) slope is ~ 0.2 MHz s⁻¹. Rùhima³ has identified several subclasses of L bursts on the basis of the sign of df/dt , which may be positive or negative or both. The slope is a function of the system III longitude of Jupiter and the modulation is therefore likely to be associated with the emission and propagation of the radiation near the planet. The lanes are quasi-periodic in time with a period of about 3 s near 21 MHz and are observed from 8 to 30 MHz (ref. 3).

Other types of lane structure have been seen in the present observations. When the time scale is expanded by a factor of 10, a second class, the fast lane (FL) bursts, is observed in which the ($f-t$) slope of modulation is near 2.0 MHz s⁻¹ (Fig. 1*a*). Again, both positive and negative $f-t$ slopes are observed. The quasi-period of the modulation is approximately 30 ms. FL bursts were observed between 5 and 17 MHz on 29 out of 56 occasions on which Jupiter bursts were recorded between April 22, 1973, and July 2, 1973. Within this frequency range they have the unusual property that the $f-t$ slope of the modulation lanes is independent of frequency (Fig. 2). At still higher time resolution a third class, the very fast lane (VFL) bursts, was observed on a few occasions both

Fig. 1 Dynamic spectra of Jupiter radio bursts. *a*, Lane bursts and fast lane bursts, *b*, very fast lane bursts, *c*, shadow drift pair burst, *d*, S bursts, *e*, U burst.



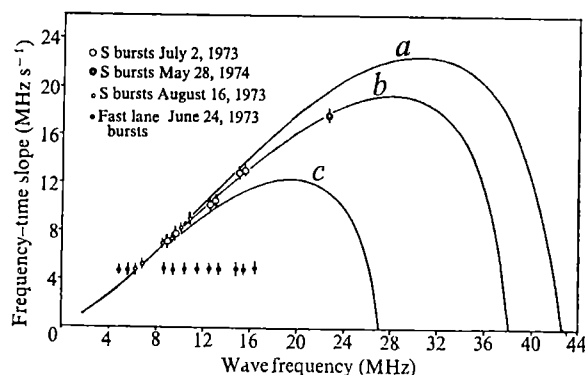


Fig. 2 Observed variation of df/dt with wave frequency for S bursts and fast lane bursts. Solid lines show the theoretical variation for cyclotron radiation from electrons of speed $0.1c$ travelling in the Io L shell for different values of equatorial pitch angle ϕ_0 . Magnetic field dipole moment $4 \text{ G } R_J^3$. a, $\phi_0 = 2^\circ$, b, $\phi_0 = 2.16^\circ$, c, $\phi_0 = 2.5^\circ$.

in the 14–17 MHz and 4–7 MHz frequency ranges (Fig. 1b). The f - t slope is approximately 15 MHz s^{-1} and is again independent of frequency between 16 and 4 MHz s^{-1} . The quasi-period of the modulation lanes for these bursts is near 4 ms.

A separate class of bursts, the shadow drift pairs, is illustrated in Fig. 1c. A typical spectrogram shows a pair of parallel dark bands with a mean separation in time of 0.4 s and a mean f - t slope of 1.4 MHz s^{-1} . They bear a remarkable resemblance to the solar radio drift pair bursts, except that they are seen in absorption rather than emission. Like these latter bursts, their properties vary only over a small range. Ten examples were recorded in July and August 1972.

Short, or S, bursts with a single frequency duration of 1 to 10 ms were observed during about half the Jupiter radio events between April and July 1973 (Fig. 1d). Their f - t slope is 10 MHz s^{-1} at 12 MHz, and is very nearly proportional to the wave frequency over the frequency range 5 to 15 MHz (Fig. 2). Their instantaneous bandwidth is typically 10 kHz, although it may be as small as 3 kHz. These measured values of df/dt are likely to be too high by approximately 0.008 MHz s^{-1} if account is taken of the effect of the analyser bandwidth⁵ on the variable frequency signal. Within the millisecond time scale of the S bursts, there are many fine structure phenomena which resemble those seen in the terrestrial vlf emissions. Triggered and periodic bursts occur frequently, and there is much evidence of wave-particle interactions from sudden changes in f - t slope where S burst spectra cross constant frequency emissions. On a few occasions, S bursts with reverse (positive) f - t slope are observed. A rare variant of the S bursts is the U burst, or inverted U burst, which shows a quasi-parabolic change and reversal in f - t slope (Fig. 1e). U bursts have been seen so far only below 10 MHz. Like the shadow drift pairs, the f - t spectra of the U bursts resemble those of the corresponding solar radio emissions, the solar U bursts.

The distribution of f - t slopes of all these bursts is shown in Fig. 3, where three main regions near 0.1 MHz s^{-1} , 1.0 MHz s^{-1} and 10 MHz s^{-1} may be seen. Similar groupings of df/dt exist for the solar radio emissions. So far no satisfactory explanation of the lane bursts has been advanced, although it has been suggested by Rùhímaa³ that the modulation results from interference between waves from multiple sources. The invariance of df/dt with f seems difficult to account for by this process. Rùhímaa also proposed, alternatively, that the lane structure is caused by the passage of the radiation through a moving spatially periodic screen after emission. Since different frequencies would be generated at different points along a field line, the frequency interval between the modulation lanes would be determined by the spatial period.

The properties of the S bursts, on the other hand, were predicted from a consideration of the likely radio emission from moving sources travelling outwards along the Jupiter magnetic field lines and radiating in the doppler shifted cyclotron mode⁶. As in the Earth's magnetosphere, the sources may be convective instabilities in almost monoenergetic electron streams⁷ or possibly isolated bunches of electrons as in the solar corona. The f - t slope is then a measure of the speed v of the source, and for a simple dipole field

$$df/dt \propto f^{4/3} A(\lambda, \lambda_m) v$$

$A(\lambda, \lambda_m)$ is zero for magnetic latitude $\lambda = 0$ or λ_m , the mirror latitude. If the magnetic dipole moment and the L shell of the electrons can be assumed, then their velocity and equatorial pitch angle ϕ_0 can be obtained from observations of df/dt over a sufficiently wide range of f . Figure 3 compares the observations with the calculated variation of df/dt with f for $v = 0.1c$ and $\phi_0 = 2^\circ$, 2.16° , and 2.5° respectively for electrons in the L shell of the satellite Io (Jupiter dipole moment assumed⁸ to be $4 \text{ gauss } R_J^3$). The mirror-point cyclotron frequency of the electrons is 38 MHz. It should be noted that if the maximum magnetic field cyclotron frequency fH_m near the planet's surface proves to be less than this, then this result may imply that the electrons are accelerated near the surface⁹. Two alternative revised models of the magnetic field⁸ derived from Pioneer 10 observations give $fH_m = 31$ and 38 MHz respectively. But fH_m is very strongly dependent on the precise location of the dipole centre and further revisions can be expected in the future.

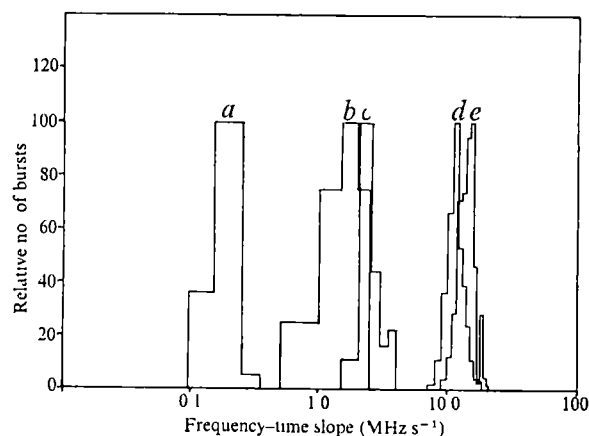


Fig. 3 Distribution of frequency-time slopes of a, lane bursts, b, shadow drift pairs, c, fast lane bursts, d, S bursts, e, very fast lane bursts. Frequency range 15–17 MHz.

The S bursts do not normally extend in frequency over more than 1–2 MHz and do not individually exhibit the variation of df/dt with f shown in Fig. 1d. Indeed, the f - t trace may be curved in either direction, not just linear. The relatively small frequency range would be expected if the radiation is from sources travelling along curved field lines and strongly beamed as expected for cyclotron radiation. Slight variations in the speed of convective beam instabilities would account for the f - t deviations. The very narrow spread in the distribution of df/dt for observations made at different times is particularly striking and contrasts, for example, with the wide distribution for type III solar bursts. It points to a source of radiating electrons of almost constant energy such as has been suggested for the model of Goldreich and Lynden-Bell¹⁰, where the satellite Io acts as a unipolar inductor and a source of constant e.m.f. across the Io flux tube as it moves through the Jupiter magnetic field.

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Alternative hypothesis for the origin of CCF xenon

I SUGGEST that the anomalous xenon isotopic composition known as carbonaceous chondrite fission (CCF) xenon is not caused by fission, but is the direct result of a modified r-process nucleosynthesis which produces an abundance peak at $Z = 54$ and the magic neutron number $N = 82$. I further propose that the xenon so produced ('R xenon') was trapped in dust grains, which were subsequently incorporated in the solar system with minimal degassing. This hypothesis also provides a natural explanation for the intimate association between R xenon and a newly discovered xenon component in primitive meteorites^{1,2}.

Analyses of the abundance and isotopic composition of xenon in meteorites have provided insight into several aspects of the early solar system and the origin of some of the complex patterns are now well understood. Recently, for example, it was confirmed that xenon in Ca-rich achondrites arises, in part, from the spontaneous fission of extinct ²⁴⁴Pu (ref 3).

One of the more enigmatic xenon compositions is found in carbonaceous meteorites and unequilibrated ordinary chondrites⁴, both of which are thought to be primitive meteorite types. It has properties which are characteristic of xenon produced from fissioning nuclides, hence its name, the CCF component.

It is still not clear whether this component actually has a fission origin, at least to the extent that the parent nuclide has not been identified. In order to identify the parent nucleus, the isotopic composition of the meteoritic xenon component must be compared with known fission spectra. Lack of identification could once have been attributed to a paucity of experimental data on fission spectra from nuclei in the mass range $240 \leq A \leq 260$, but this is not now the case⁵. A comparison of all presently known fission spectra with the meteoritic composition does not, however, yield any match (D C B, unpublished).

Failure to identify the hypothetical parent of the xenon component has not given rise to alternative interpretations but, rather, has generated more exotic suggestions as to the parent⁶.

Anders and Heymann⁶, and more recently Takaoka⁸, have shown that the amount of R xenon in a meteorite correlates with the abundance of trapped ¹³²Xe. This correlation is generally interpreted as a statement that the 'parent' nuclide which fissioned to give rise to R xenon was a volatile element⁶. There are, however, other interpretations, which I shall discuss later. The calculated absolute abundance of R xenon depends on the assumed trapped xenon isotopic composition, but is generally characterised by ¹³⁶Xe concentrations of $\sim 0.1 \times 10^{-10}$ to 1.0×10^{-10} cm³ STP g⁻¹.

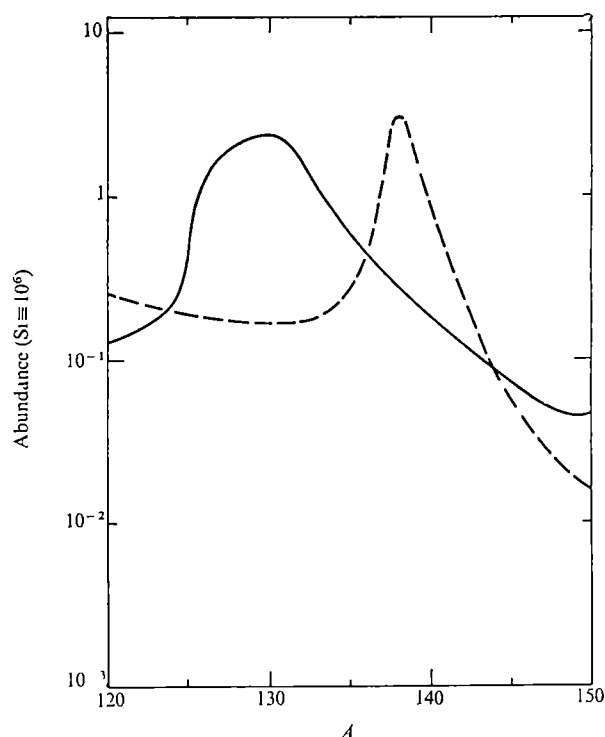


Fig 1 Schematic representation of the abundance curves expected from 'classical' r-process (—) and s-process (---) nucleosynthesis in the mass range $120 \leq A \leq 150$. Data from ref 20

The most recent estimate (ref 9 and R O Pepin, private communication) of R xenon is given in Table 1. This estimate differs from previous ones^{10,11} primarily in that the ¹³²Xe/¹³⁶Xe ratio is lower (0.22 compared with 0.37) but is within the uncertainties associated with the earlier estimates. The new estimate agrees with that given by Marti¹² for ¹³⁶Xe, ¹³⁴Xe, ¹³²Xe.

As pointed out by Phinney¹ and Manuel *et al*², analyses of xenon isotopic compositions evolved from carbonaceous meteorites during stepwise heating experiments reveal the existence of an isotopically distinct xenon component which is characterised by a progressive enrichment (¹²⁴Xe > ¹²⁶Xe) in the isotopes of xenon which are shielded from contributions by way of fission. These workers have shown that the new component ('P xenon') does not arise from *in situ* nuclear processes and that it seems to be fairly uniformly mixed with R xenon. Manuel *et al* designate the mixture of P xenon and R xenon as Component X. More recent studies¹³ indicate that P xenon may be present in most primitive meteorites. Manuel and his co-workers do not speculate in detail as to the origin of P xenon, but they do suggest that P xenon and R xenon 'result from a common source'. In this regard, it is worth noting that Phinney attributes P xenon to p-process nucleosynthesis.

There is increasing evidence that some of the particulate matter (dust grains) in the primitive solar nebula were not heated sufficiently to degas quantitatively or chemically equilibrate with the bulk of the nebula, and have retained in part the elemental and isotopic characteristics of a period which predates the formation of the solar system. I shall designate such isotopic compositions as 'non-solar'. The first evidence was the discovery of a singular neon isotopic composition in carbonaceous meteorites¹⁴, characterised by a trapped ²⁰Ne/²²Ne ratio ≤ 3.4 , which was suggested to be 'non-solar'¹⁵. Subsequently, analyses of oxygen isotopic ratios in carbonaceous meteorites have led to the discovery of an isotopic anomaly which has also been described as 'non-solar'¹⁶. Work by Gray and Compston¹⁷ and Lee and Papanastassiou¹⁸ has revealed the

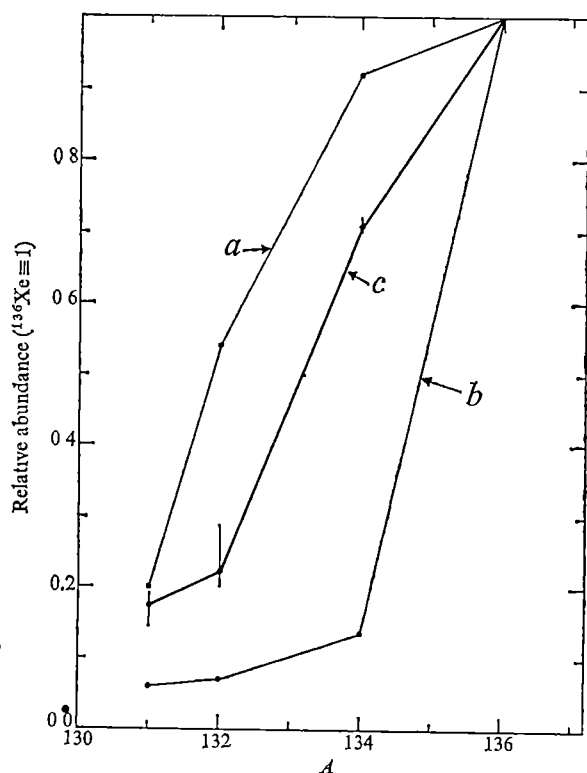
Table 1 Isotopic composition of R xenon

^{131}Xe	^{132}Xe	^{134}Xe	^{136}Xe	Ref.
—	0.28	0.71	$\equiv 1.0$	12
0.17 ± 0.02 -0.03	0.22 ± 0.07 -0.02	0.71 ± 0.01	$\equiv 1.0$	9 and R O Pepin, private communication

presence of magnesium isotope anomalies, again in carbonaceous meteorites, which, too, have been interpreted as being 'non-solar'. Theoretical models of primitive solar nebulae¹⁹ and the collapse of rotating protostars (D C B and P Bodenheimer, to be published) indicate that at heliocentric distances $\gtrsim 1.5$ AU, nebular temperatures would not have been sufficient to degas quantitatively interstellar dust grains present in the primitive nebula. Thus, the available evidence indicates that a fraction of the solid matter in the early solar system retained the elemental and isotopic compositions characteristic of their place of formation.

Generally, r-process nucleosynthesis is imagined to imply the rapid addition of neutrons to nuclei to synthesise elements beyond the iron peak. Typically, one is concerned with neutron densities $n_n \sim 10^{24} \text{ cm}^{-3}$ and temperatures $\sim 10^9 \text{ K}$. Under such conditions, the time scale for the addition of neutrons, $\tau_n = (n_n v_T \sigma_n)^{-1}$ where v_T is the mean thermal speed of the neutrons at temperature T and σ_n is the capture cross section of a given nucleus for neutrons, is much less than the time scale (τ_β) for β^- decay of the nucleus. In this situation, equilibrium is established between (n, γ) and (γ, n) reactions, with β^- decay providing a small perturbation or 'leakage' to the equilibrium established by the neutrons and photons. Such a picture leads to an abundance curve with a peak at mass 130 (ref 20), shown schematically in Fig 1. The peak is associated with a magic number ($N = 82$) of neutrons.

Fig. 2 Relative abundance ($^{136}\text{Xe} = 1$) of the xenon isotopes ^{134}Xe , ^{132}Xe and ^{131}Xe . a, b, Curves for r-process and s-process, respectively, obtained by renormalising those in Fig 1 so that their abundance peaks occur at mass 136, rather than mass 130 and 138 respectively. c, Composition of R xenon.



In the other limit, $\tau_\beta \ll \tau_n$, nucleosynthesis (s-process) proceeds along the valley of β -stability. Again, one finds that the effect of the closure of shells containing magic numbers of neutron appears in the form of a peak in the abundance curve at 82 neutrons and a mass of 138 amu (ref 20) (Fig 1). Note that both abundance peaks are steep and smooth because of the smooth variations in nuclear properties near the magic number of neutrons.

Figure 2 shows the abundance curves one would expect if the r-process and s-process peaks occurred at $A = 136$ rather than $A = 130$ and 138 respectively. The curves are normalised so that $^{136}\text{Xe} = 1$. Also shown is the abundance curve defined by R xenon. The R xenon curve falls between r-process and s-process abundance curves at all isotopes. If the r-process was altered to give rise to a peak at $A = 136$, it would be steeper than the plotted r-process abundance curve. In the limit, it must converge to the s-process abundance curve. Thus on a qualitative basis, the isotopic structure of R xenon is consistent with an r-process nucleosynthesis which produced an abundance peak at $A = 136$.

The physical condition required to shift the r-process abundance peak to larger values of A is basically that τ_n be increased. This allows the β^- decay to proceed further up the magic neutron ladder, at $N = 82$, than is the case for the 'standard' r-process nucleosynthetic chain. In fact, ^{136}Xe lies on the β^- decay ladder at $N = 82$ and is the first stable nucleus encountered by the ladder. The increase in τ_n can be brought about by lowering n_n and/or T . Preliminary estimates of the range in n_n and T required suggest a low density, high temperature regime such as one might encounter in a shock wave traversing the atmosphere of a supernova precursor.

It has been suggested²¹ that many of the p-process (proton-rich) nuclei are created in a supernova shock. If so, an intimate mixing of p-process nuclei and R xenon might result. The newly discovered evidence for such a mixing in carbonaceous meteorites would be a natural consequence of the modified r-process hypothesis.

One might expect manifestations of the modified r process in other elements. In particular, effects should be seen at three of the isotopes of krypton, namely ^{86}Kr , ^{84}Kr , and ^{83}Kr . As with ^{136}Xe , ^{86}Kr is the first stable nucleus encountered by a β^- decay ladder at a magic neutron number ($N = 50$). The magnitude of the attendant krypton effect cannot be estimated without more detailed calculations.

The abundance ($\text{cm}^3 \text{ STP g}^{-1}$) of R xenon does not lead to any particular problem (as is the case for the fission hypothesis). Assuming that the concentration of ^{136}Xe (R xenon) in dust grains is similar to typical xenon concentrations in meteorites ($\sim 10^{-8} \text{ cm}^3 \text{ STP g}^{-1}$), only $\sim 1\%$ of the material in carbonaceous meteorites need contain R xenon to explain the observed bulk abundance data. The correlation of ^{136}Xe (R xenon) with trapped ^{132}Xe would be expected if dust grains which contain R xenon mix with varying amounts of dust grains formed in the solar nebula. A similar correlation exists between trapped ^{36}Ar and the singular neon component discussed previously¹⁵, and has been interpreted in the same manner as the ^{132}Xe R xenon correlation^{15,22}.

Perhaps the most obvious future theoretical effort would be to vary the r-process nucleosynthesis parameters to give rise to an abundance peak at ^{136}Xe , and compare the resulting spectrum with that of R xenon. It is likely that requiring the abundance peak to occur at ^{136}Xe is a necessary, but not sufficient constraint on the nucleosynthetic calculations. One would like to determine the sensitivity of the calculated spectrum to variations in the controlling physical parameters (T , n and so on). If, within the uncertainty in the calculated spectrum, agreement is obtained between the experimental and theoretical spectra, one would like to know the astrophysical environment, or range of environments, likely to engender R xenon. In this connection, will the same environment(s) give rise to P xenon?

The experimental data available to date have primarily come

from stepwise heating experiments on bulk samples. This type of experiment provides a quantum jump over simple bulk analysis in the detailed knowledge one obtains from a sample. The state of the art now, however, requires a further quantum jump in information content per experiment, namely stepwise heating experiments on various discrete mineral phases or assemblages from a meteorite. It would seem particularly important to do rare gas analyses of at least neon and xenon in a given sample, and to have oxygen and magnesium isotope analyses done on aliquots of the rare gas samples. The questions are: do R xenon, neon E (ref 15) and the oxygen and magnesium isotope anomalies occur together, with what frequency and in what minerals? Are P xenon and R xenon well mixed, and if so is it on a microscopic scale (individual mineral phases and/or grains) or a macroscopic (in bulk) scale?

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The beginning of a new cycle of solar activity

ON November 15, a high latitude sunspot marked the beginning of a new cycle of solar activity. The present cycle is on the decline and is approaching its minimum, a new cycle thus starting before the old one has completely disappeared. At this time the sunspots belonging to the old cycle originate near the equator, whereas those of the new one occur at higher latitudes.

The latitude of the sunspot observed was +37°, and it was 60° from the central meridian. It could be seen clearly the following day but not on November 17, when it crossed the western limb. The first spots of a new cycle usually appear more than a year before the coming minimum, so because the present activity is still relatively high, the minimum cannot be expected to occur before 1976.

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Mathematical prediction of climatic change

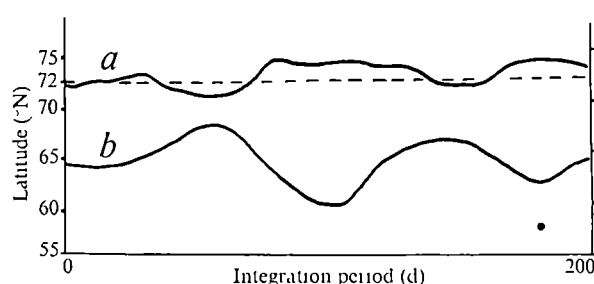
A WIDESPREAD and increasing degree of attention is being paid to the problem of understanding the physical factors which lead to climatic change. Analysis of past weather data¹ derived from a wide variety of sources has shown how the main characteristics of climate have changed over recent centuries. If, however, scientific theories are to be developed, it is desirable that mathematical models of climate be constructed. These must include at least the primary global scale dynamics of the atmosphere and should allow the operation of necessary long time-period integrations on available computers.

The primary physical factor responsible for initiating the stirring of the atmosphere into its ever changing assembly of the cyclonic waves and anticyclonic systems is the meridional distribution of solar radiation, $S(\theta)$, where θ is the latitude. The response of the atmosphere to a given $S(\theta)$ and to the associated requirement of balancing the heat budget is seen in the continuously created train of weather systems which strive to rectify the imbalance, these systems are important agencies in determining climate. The sensitivity of climate to changes in solar radiation or changes in atmospheric transparencies should, therefore, be investigated.

In approximate calculations Budyko² used a system of empirical equations describing an assumed static balance (in a temporal sense) between the residual vertically integrated and zonally averaged annual radiation heating at a given latitude and the vertically integrated meridional flux divergence of heat. He assumed that his empirical heating functions were still valid when the total solar radiation was altered by a series of small percentages. An iterative computer procedure allowed Budyko to calculate the associated shift in the meridional distribution of mean atmospheric temperature, atmospheric and surface albedo, and the new mean, zonally averaged, simulated ice edge position. The primary atmospheric physics on a global scale is contained in Budyko's model (although rather crudely) but he assumed a static atmospheric balance, purporting to represent an asymptotic, annual average situation, corresponding to a prescribed solar heating, and there is no indication of how sensitively the calculated results depend on the mathematical forms of the empirical functions used. Consequently, his main result, suggesting that a 1.5% reduction in incoming solar radiation (or an equivalent change in atmospheric transparency) would lead to a new ice age, has met with scepticism. An extended form³ of the calculation has indicated that as much as a 5% reduction in solar radiation would be needed before large scale movement of the ice edge would occur.

These simplified static calculations, however, tell us nothing about time variations around the mean values of the large scale structure of atmospheric dynamics, indeed it may be completely incorrect to assume a stable balance of the averaged flow system for a perturbation of solar heating greater than some

Fig 1 Position (latitude) of the zonally averaged simulated ice edge as a function of time over a 200-d period. a, Solar heating values representing the present decade; b, a 3% reduction in solar heating.



critical value. For example, with small reductions in solar heating the zonally averaged ice-snow edge in the model may oscillate about its mean latitudinal position with a small spatial amplitude, as in a dynamically stable state, but with larger reductions the amplitude may become larger and there may occur a progressive movement towards the equator of the zonally averaged snow-ice position.

We have developed a time-dependent mathematical numerical model containing only the global scale dynamics, which simulates observed oscillations of the total atmospheric eddy kinetic energy associated with variations in the intensity of cyclonic systems. Basically, it is a modification of that introduced by Phillips⁴, and used⁵ in studies of the dynamics of anticyclonic blocking. The effects of standing waves on the planetary scale created by mountain barriers are not included and the effects of heating and friction in the model are taken as zonally averaged over continents and seas. The heating function, however, is not fixed geographically, as in the previous model, but is dependent on model atmospheric temperature, and a surface thermal balance equation is used, which predicts model surface temperatures. The very long time period integrations essential to theoretical studies of climate are practical from a computing point of view: only 10 min computing time is required to carry out approximately 150-d integrations on the Harwell IBM 360/195 computer. The results obtained predict a scientifically interesting characteristic of the dynamics of climatic change and also show that numerical experiment can be applied profitably to this important practical question.

The model uses an initial meridional distribution of solar heating, $S(\theta)$, equivalent to annually averaged and zonally averaged observed heating values. After a long integration period of about 300 d, the model apparently reaches an asymptotic 'equilibrium' dynamic state with the simulated snow-ice edge oscillating about a mean latitude of roughly 72°N. This simulation is based on the predicted model surface temperature, and includes an associated albedo distribution taken from observational data, an empirical time lag could have been built into the representation but temporal variations in the position of the edge are slow compared with the time step of integration. As such a representation would have involved further extensive computing it has therefore been omitted at this stage. The moving position of the edge is shown in Fig. 1 for a 200-d period. At the end of that period S was reduced by 0.5% and the computation continued for a further 400-d period. The calculated mean position of the ice edge over that period coincided with the result obtained previously using our statical method³. The process was repeated for successive small reductions, δS , in S . When δS approached 3% the westerly, mid-latitude, cyclonic wave type of flow became punctuated increasingly by low latitude, Hadley-cell circulations which penetrated to higher latitudes for increasing periods of time. Indications of this characteristic of climatic change have already been noted by Lamb¹. At the same time, the spatial amplitude of the oscillating edge increases sharply as δS approaches 3% (Fig. 1) although the mean position is at about the latitude predicted by statical calculations³. In the type of model used by Everson and Davies, latitude does not correspond exactly to latitude on the spherical Earth although there is a close approximation.

We deduce from these results that, although the computed movement of the zonally averaged ice edge follows roughly the results of previous statical calculations³, the dynamic model shows signs of rapidly increasing instability (reflected in the increasing amplitude of oscillation of the simulated ice edge) at each mean equilibrium position, as solar heating is reduced by up to 3%. The model is known not to reproduce the low latitude (Hadley cell) mean meridional circulations very well and several important feedback processes are omitted, there is a pressing need to study the dynamics of climatic change with more sophisticated model systems including a realistic oceanic-atmospheric link and representations of mean seasonal movements. Our initial results form, however, a step forward

from previous calculations based on statical models, and show that, although equilibrium solutions (of the Budyko type) exist with as much as 5% reduction in solar heating, their stability becomes very suspect as 3% reduction values are approached, and the atmospheric circulation changes fundamentally. This suggests that there is a danger that a further increase will trigger off a progressive movement of the snow-ice edge towards the equator. Calculations beyond this stage have not been carried out, as we feel that the premises of the model are unlikely to apply.

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Detection of elastic strainfields caused by fault creep events in Iran

SLIP movements along active faults may either occur violently, and generate earthquakes, or gradually in a little understood process called fault creep¹⁻⁵. An interesting feature of fault creep is that it is frequently episodic rather than continuous and the episodes of movement propagate along the fault¹. The mathematics, but not the physics, of the process are identical to those of crystal dislocations⁶.

Although displacements across faults have been monitored carefully for some time^{4,5}, there has been almost no study of the transient strains that must be associated with the passage of each creep event. Frank⁷ has discussed the strain-time histories that can be expected theoretically during typical strike-slip creep events, and we here follow his formulation.

For a single, infinitely long, edge dislocation of slip strength, b , normal to the surface in a homogeneous semi-infinite half-space⁷

$$e_{\phi} = \frac{-b}{4\pi y} \left\{ (1-2\nu) \frac{1}{1+X^2} + (1+2\nu) \times \left[\cos 2\phi \frac{2X^2}{(1+X^2)^2} + \sin 2\phi \frac{X(1-X^2)}{(1+X^2)^2} \right] \right\} \quad (1)$$

where ν is Poisson's ratio, e_{ϕ} is the linear strain at a point x, y , $X = x/y$, and the surface trace of the fault lies along the x -axis with y chosen such that in a right-handed coordinate system z is positive upward, ϕ is the angle between strainmeter and the x -axis, and increases in an anticlockwise fashion looking down.

A screw dislocation parallel to the x axis at a depth d gives a strain of

$$e_{\phi} = -\frac{b}{4\pi y} \frac{4D}{1+D^2} \sin\phi \cos\phi \quad D > 0 \quad (2)$$

$$= 0 \quad D \leq 0$$

Where $D = d/y$. The function $-b/4\pi y$ indicates that the strain magnitude is proportional to the size of the dislocation and decays inversely with distance from the fault.

Equations (1) and (2) describe the spatial variation of strain resulting from static dislocation. If, however, the dislocation moves with a velocity which is slow compared with the shear

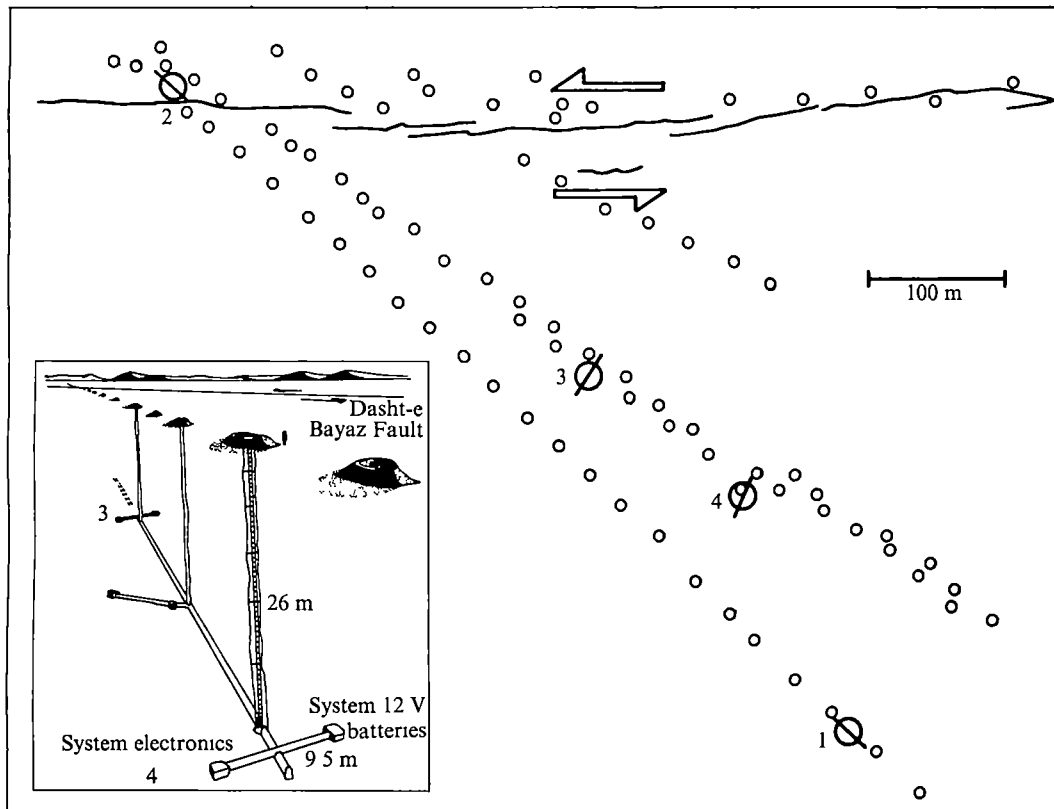


Fig. 1 The location of the strainmeters superimposed on a map of the Dasht-e-Bayaz fault break⁸. Open circles, vertical well shafts connecting qanats to the surface, 1-4, positions of strainmeters—the symbols indicate orientation. The insert shows a diagram of the qanat system containing instruments 3 and 4.

wave velocity, the strain as a function of time may be obtained by substituting

$$X = (x_0 - ut)/y$$

in equation (1) and

$$D = (d_0 - wt)/y$$

in equation (2). The parameters x_0 and d_0 refer to the position of the dislocation at time, $t = 0$, and u and w are the x and z components of the velocity. As equation (1) and (2) are not invariant with respect to a reversal in the velocity, the strain as a function of time may be used to determine the direction of propagation of a dislocation.

The instruments on which we have observed creep related strains are situated in eastern Iran near to the fault break⁸ of the 1968 Dasht-e-Bayaz earthquake. They are sited in horizontal underground irrigation tunnels called qanats (Fig. 1). These connect to the surface at regular intervals through vertical well shafts. Instruments 3 and 4 are installed in a completely separate qanat system (insert, Fig. 1) from the array comprising instruments 1 and 2. Within an array the instruments share the power supply and the recording system, but there is no electrical connection between separate arrays. These circumstances greatly reduce the possibility that a freak instrumental effect will be observed.

Column *a* of Fig. 2 shows data from the four instruments. Instruments 1 and 2 were recorded on one chart paper and 3 and 4 on a second. To record the data (Fig. 2*a*) the instruments use pressure-sensitive paper marked by a galvanometer needle that is struck every 10 s by a chopper bar. The sampling interval for instruments 1 and 2 is much longer than that for instruments 3 and 4 which gives rise to discontinuous traces (Fig. 2*a*). Tides are visible on the records and long spans of tidal data from instruments 1 and 2 and a third now defunct instrument in the same qanat have been analysed. The results (Fig. 2*a* and *b*), indicate that no significant rigidity anomalies are apparent in the region of the arrays. The very large strain magnitude observed on instrument 2 (Fig. 2*b*) excludes the possibility that a pressure effect has

been observed⁹. The temperature coefficients of the instruments are less than 10^{-7} per °C and the temperature in the instrument 2 site remains constant to within 0.1°C over periods of days. The excursion is therefore too large and too rapid to be a thermal effect.

Predicted strain signals for an edge dislocation—equation (1)—propagating eastwards ($u = 200\text{ mm s}^{-1}$, Fig. 2*c*), and westwards ($u = -200\text{ mm s}^{-1}$; Fig. 2*d*) along the fault, show clearly that an eastward, but not a westward, propagating creep event fits the data rather well. That direction of propagation is also suggested by the fact that the event first appears on instrument 2. Furthermore, the velocity of 200 mm s^{-1} both produces strain records like those observed, and accounts for the different onset times on the different instruments.

The strain records for instruments 3 and 4 for 12 h before the observation of the events described here, are significantly disturbed from the normal tidal signal for the same instruments (Fig. 3*a*). For comparison, a short section of typical tidal data for instrument 3 is shown (Fig. 3*b*). Consideration of the predicted strain record (Fig. 3*c*) for these instruments for a downward propagating (equation (2) $w = -200\text{ mm s}^{-1}$) screw dislocation suggests that some of the signal observed by instruments 3 and 4 in this 12-h period might have been produced by downward propagating creep events. These events are not, however, clearly visible on the records of instruments 1 and 2 possibly because of the high noise level or the long sampling interval.

The present network of four instruments is insufficient to permit determination of the depth and extent of the creeping surface. The event interpreted as having been caused by an edge dislocation was, however, detected to the west of instrument 2 and was still visible until it was to the east of instrument 1. It must, therefore, have propagated more than 800 m. It is of particular interest that this same event can be explained by an infinite dislocation with a slip strength, b , of less than $10\text{ }\mu\text{m}$. If the depth of the edge dislocation is limited by allowing it to generate or consume a trailing or leading screw dislocation, the size of the required slip and the form of the predicted signals are not altered significantly, unless the whole process is restricted to a few tens of metres near the surface. That is

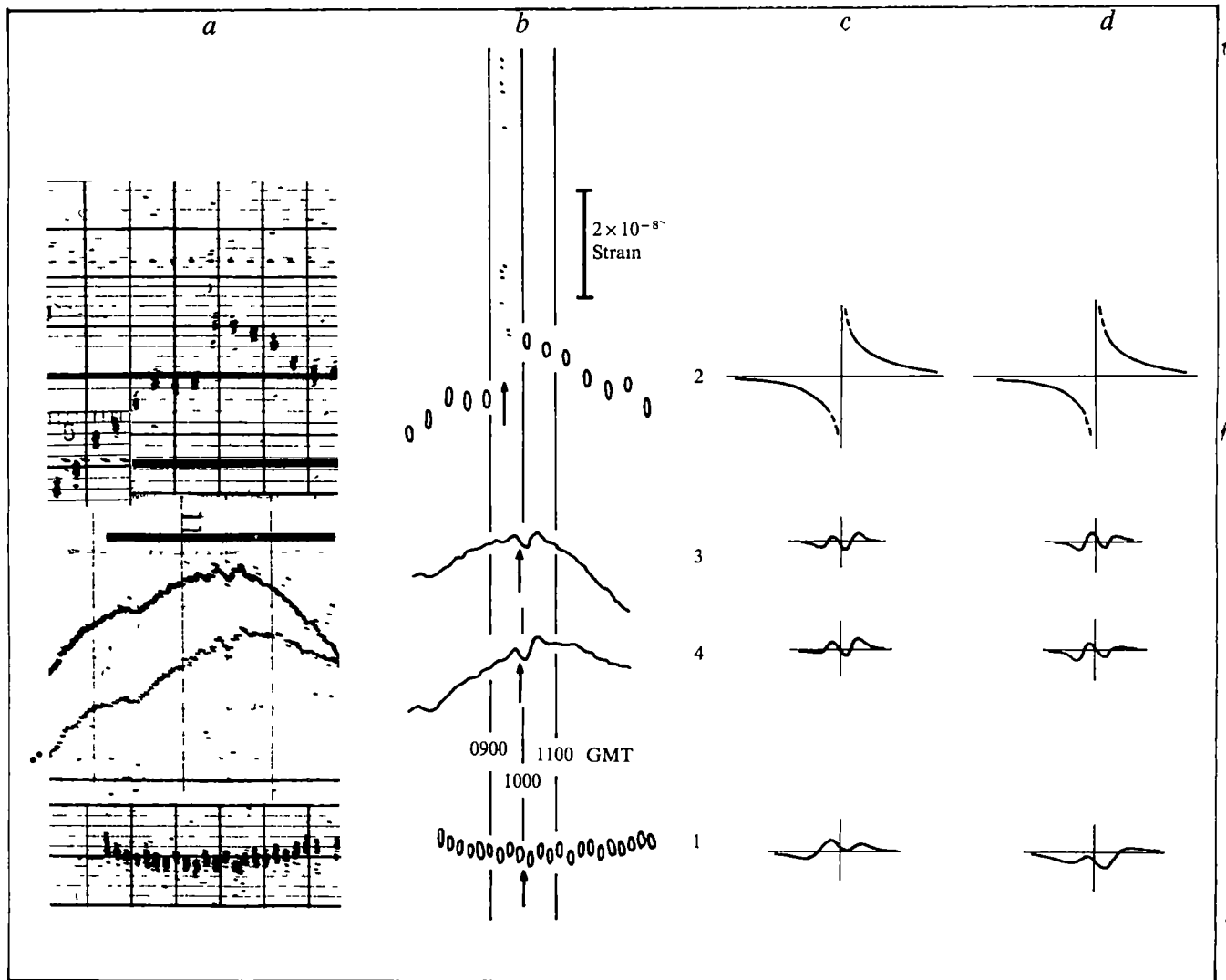


Fig. 2 *a*, Data recorded by the strainmeters between 0900 and 1000 on April 24, 1974 (the numbers in the centre of the figure correspond to the numbers of the instruments). Traces 1 and 2 were written by an earlier and noisier design of strainmeter than were traces 3 and 4. *b*, tracings of the data shown in column *a*. The large excursion of the trace of instrument 2 (which is closest to the fault) is easily visible on the original record, though not on the reproduction in column *a*. *c, d*, Predicted strain records (*c*, for an eastward propagating edge dislocation, *d*, for a westward propagating edge dislocation). The vertical lines in columns *c* and *d* correspond to $X = 0$ in equation (1), and the arrows on the data tracings (column *b*) correspond to where the vertical lines overlay when *c* is fitted to *d*. The horizontal scales are everywhere the same. The velocity of the creep event in *b* and *c* is 200 mm s^{-1} . The vertical scales are the same and correspond to the scale bar. The amplitudes of the predicted signals have been chosen to facilitate comparison with the data. They are within $\pm 20\%$ of the amplitudes predicted by a dislocation of slip strength, $b_s = 10 \mu\text{m}$ except in the case of instrument 1 which, for clarity, has been exaggerated by a factor of 2.

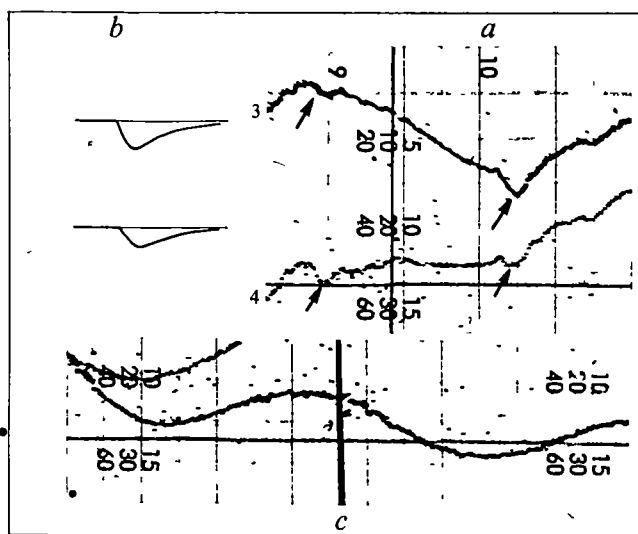


Fig. 3 *a*, Records from instruments 3 and 4 for 12 h before the data displayed in Fig 2, *b*, predicted form for downward propagating ($w = -200 \text{ mm h}^{-1}$) screw dislocations, *c*, predicted form for a typical span of tidal data recorded at the same site on instrument 3.

clearly unlikely for an event which propagated at least 800 m. Models which invoke exotic departures of rigidity from an homogeneous half space are excluded by the consistency of the tidal data.

Although a creep event was previously observed on the same fault by a creepmeter 5 km from the strain array, and even though geodetically measured movement is believed to be occurring (J Brander, personal communication), no creep events were detected in association with the strain signals. That is hardly surprising in view of the fact that 10 μ m is well below the threshold level of our own and other people's creepmeters (Californian creep events are often 10³ times larger).

A further point of interest is that our predicted signals do not resemble those of typical creep events as written by creepmeters. In that respect they differ from strain records obtained at Stone Canyon¹⁰. Though it is possible that those records were produced by very localised fault creep¹⁰, or by local slumping, all of our strainmeter designs have produced records that resemble creep events on creepmeters. Our instruments have been installed at stable sites in England and we believe that these 'events' are caused by instrumental or siting problems because they usually occur randomly on closely grouped instruments.

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Lineations in the Magnetic Quiet Zone of the North-west Atlantic

A MAGNETIC and seismic reflection survey in the Magnetic Quiet Zone shows that basement topography and magnetic anomalies are lineated parallel to the boundary of the quiet zone. Two narrow zones of reversed polarity are identified. The results imply that the zone boundary is an isochron separating a period of seafloor spreading in which rapid geomagnetic reversals occurred from a period in which few reversals occurred.

There have been many attempts to explain the presence of the Magnetic Quiet Zone which lies seaward of the continental shelves bordering the North Atlantic. In terms of crustal layer thickness and basement topography the quiet zone resembles normal oceanic crust. It is, however, unusual because the magnetic anomalies have a low ampli-

tude within the area. The following explanations for the attenuated nature of the anomalies have been suggested: (1) magnetisation during a time interval characterised by the absence of geomagnetic polarity reversals¹⁻³, (2) original magnetisation with very low inclinations acquired in equatorial magnetic latitudes during the Triassic⁴, (3) hydrothermal alteration resulting in reduced magnetisation⁵, and (4) intrusives rather than extrusives as the dominant component of layer 2 (ref 6). These have been reviewed by Pitman and Talwani⁷.

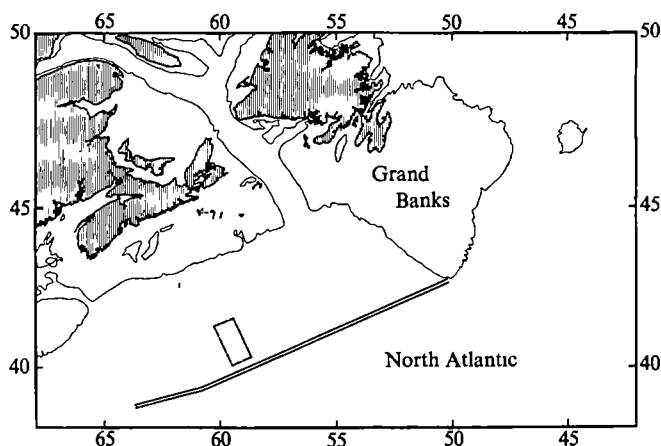


Fig. 1 Location chart showing the area of the survey (dashed lines) and the quiet zone boundary (double solid lines).

In 1972, detailed magnetic, seismic reflection and sonobuoy seismic refraction studies were carried out within an area 150 km × 70 km in the quiet zone of the North-west Atlantic (Fig 1). The purpose of the experiment was to test the hypotheses concerning the nature and origin of this area. The measurements were made from CSS Hudson at a track spacing of less than 7 km (Fig 2). Using LORAN-C in the range-range mode together with satellite navigation, the survey lines could be positioned to an accuracy of 200 m. The observed magnetic anomaly field was compared with a field calculation based on a layer of constant thickness with the observed topography of oceanic basement, so that the presence of magnetic reversals, if any, could be detected. The results provide estimates of the magnetisation of layer 2 in the quiet zone and indicate the direction of lineation of the magnetic anomalies and of topography of the top of layer 2.

Many of the experimental methods used in this study have been described previously⁸. The magnetic data were corrected for diurnal variations which are of the same amplitude, approximately 60 gamma, as the spatial variations of the magnetic anomaly field. A magnetometer manufactured by Sander Geophysics was moored in the survey area and the data from this and observations at the magnetometer station at Bedford Institute, Dartmouth, Nova Scotia, were used to make the diurnal corrections.

During the survey no unusual magnetic disturbances occurred and the r.m.s. crossover error of the corrected total magnetic field is 4.5 gamma. The anomaly field was obtained by subtracting the 1965.0 IGRF regional magnetic field⁹ plus a constant shift of 173 gamma. The latter is necessary if a mean value of zero for the anomaly field¹⁰ is desired. The magnetic data were then contoured and the resulting contour chart is shown in Fig 2.

The seismic reflection data were collected along fewer tracks than the magnetic measurements (Fig 2). Oceanic

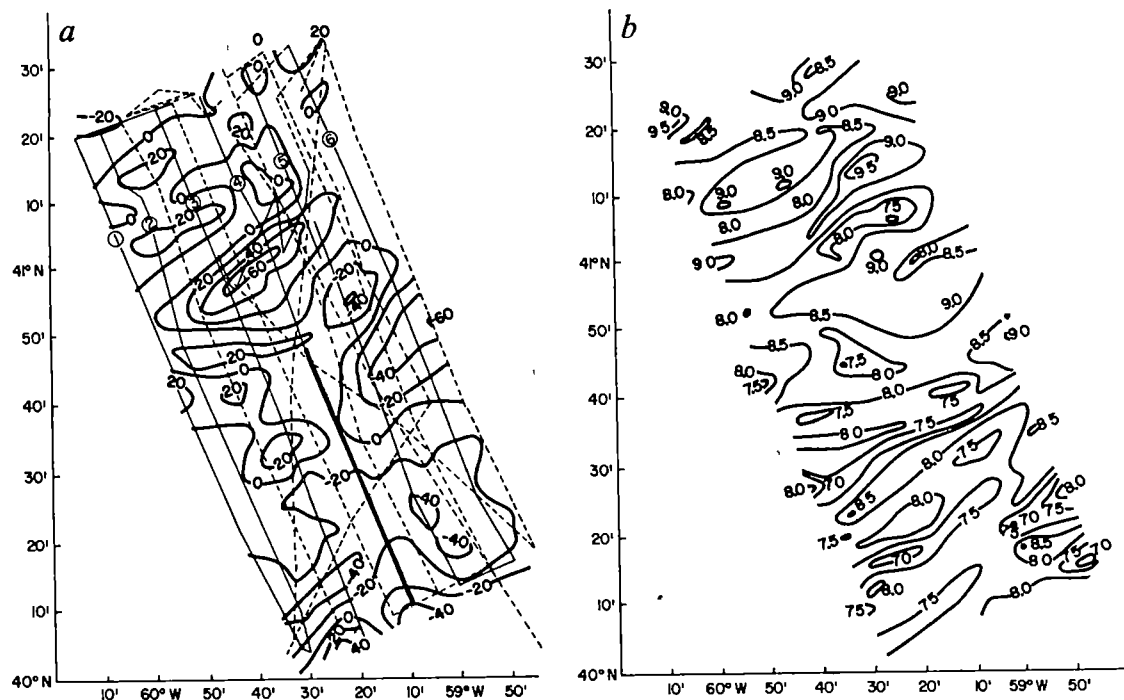
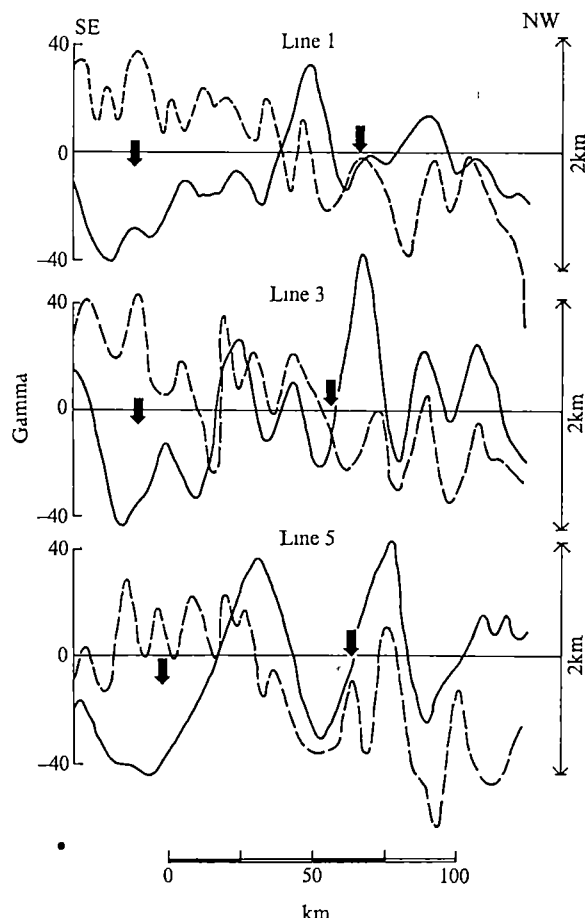


Fig. 2 Contour charts *a*, the magnetic anomalies, *b*, the basement topography, showing the tracks along which data were collected ---, Tracks along which only magnetic data were collected, —, tracks along which magnetic and seismic reflection and refraction data were obtained. The circled numbers on the solid lines are line identification numbers, used in Fig. 3. The magnetic contour values are given in gamma and the topography values are in km below sea level.

Fig. 3 The magnetic anomalies (—) and corresponding basement topography (---) along three lines. The line numbers are those indicated in Figure 2. The arrows denote positions at which there is a negative correlation between basement topography and the magnetic anomalies.



basement was invariably observed although in some instances it was difficult to determine accurately the top of this layer because of the rough nature of the reflector which caused side echoes. The depth to basement in one-way travel time was converted into distance using a mean velocity of 2.0 km s^{-1} for the sedimentary column down to horizon β and 3.0 km s^{-1} for the remainder of the section. This velocity is based on the sonobuoy results.

The contour maps of the magnetic anomalies and of the basement topography show that both are lineated approximately parallel to the quiet zone boundary (Figs 1 and 2). The magnitude of the total magnetic anomaly field varies from about 60 to -40 gamma, and wavelengths of the prominent anomalies are about 30 km, which is similar to the wavelengths of anomalies within the disturbed zone. With respect to its lineated appearance and its relief of 1 km or more, the character of oceanic basement is similar to the character of the exposed basement rocks near the crest of the mid-Atlantic ridge. This suggests a seafloor spreading origin for the quiet zone. The magnetic lineations could be a reflection of the basement trends and could also be influenced by geomagnetic reversals.

In interpreting the results, answers to three main questions concerning the quiet zone were sought.

First is there evidence that geomagnetic reversals have occurred and, if so, how frequent are they? Second, what is the magnetisation of oceanic basement? Third, was the oceanic crust formed at low geomagnetic latitudes?

To detect the presence of reversals, correlations of the magnetic anomalies with topography were made. Figure 3 shows the basement topography and magnetic anomalies along three tracks which cross the survey area. The topographic profiles show more variation than the magnetic anomalies. The attenuation of the shorter wavelength components of the magnetic anomalies is caused by the large separation (about 8 km) between the magnetic source and the level of measurements. Figure 3 indicates that the topography and the magnetic anomalies correlate well in terms of the positions of the peaks and troughs and suggests that

most of the magnetic anomalies may be caused by basement topography. But at two locations on each of the profiles there is a negative correlation. At the positions shown by the arrows in Fig 3 the basement relief is insufficient to cause the magnetic lows. This is consistently observed on all of the NW-SE tracks. So there are two strips trending parallel to the magnetic lineations where magnetic data and basement topography suggest that the basement rocks must possess reversed polarity. The good correlation between topography and magnetic anomalies elsewhere shows two significant characteristics. First, the region is predominantly underlain by oceanic basement with remanent magnetisation in the normal direction, and second the larger part of the anomalies must be caused by variations in basement topography.

A theoretical model of oceanic basement describing the topographical variations within the survey area was constructed using rectangular blocks, $1.8 \text{ km} \times 5.4 \text{ km} \times 1.8 \text{ km}$ thick. This thickness has been used by others⁴, so comparisons of results can be made easily. The blocks were aligned so that the 5.4 km length lay in the direction of the lineations and the 1.8 km length perpendicular to it. The heights of the blocks were raised or lowered to simulate the observed topography. Calculations of the magnetic field arising from this three-dimensional model were made for various values of magnetisation, magnetic inclination and strike, and for various widths of the zones of reversed polarity. With these results, an estimate of the magnetisation of the basalts can be obtained.

We computed the three dimensional magnetic anomalies using declinations and inclinations consistent with both Jurassic and Triassic palaeomagnetic pole positions for North America. A remanent magnetisation value of $1.1 \times 10^{-2} \text{ emu cm}^{-3}$ gave the best fit in the zones of normal polarity and for the sake of simplicity in the model, the same value was used in the two zones of reversed polarity shown in Fig 3. In the latter case, the high magnetisation value imposed a limit on the width of the reversed zones in the model of 1 km. A more detailed model with lower values of reversed magnetisation would undoubtedly allow the reversed zones to be wider, particularly in the southernmost zone. Simplified contour maps of the computed magnetic

anomalies for the Triassic and Jurassic models and for the observed data are presented in Fig 4. These were obtained by shading regions above 20 gamma and below -20 gamma. Although the correspondence between the observed and computed maps is not exact, the main belts of positive and negative anomalies appear in each. The Jurassic model gives the best fit to the observed anomalies in the northern part of the area where reversals do not affect the magnetic anomaly pattern. This is additional evidence to support the argument that low magnetic latitudes during the Triassic are not required to explain the magnetic anomalies in the quiet zone and further suggests that the crust is Jurassic in age. Although the models could be varied to resemble the observations more closely, the numerous geological variations which have not been included in the model together with the errors in the measurements make these refinements unrealistic.

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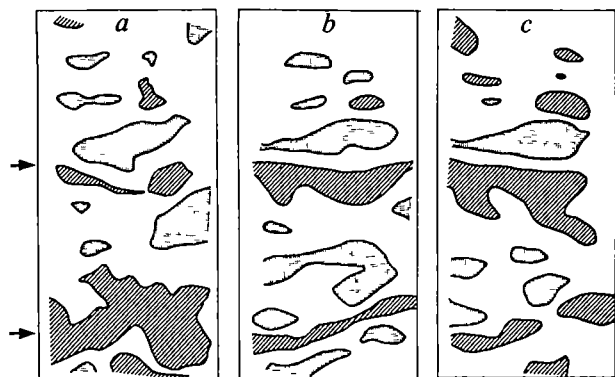
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Fig 4 Simplified contour charts of the magnetic anomaly field as observed (a), calculated from the Jurassic model (b) and the Triassic model (c). Anomalies greater than 20 gamma are shown in the dotted areas and anomalies less than -20 gamma are shown by dashed areas. For the Jurassic model a magnetic inclination of 60° and declination of 0° were used. For the Triassic model the values were 20° and 20° respectively. The remanent magnetisation was $1.1 \times 10^{-2} \text{ emu cm}^{-3}$ for both models. 1 km zones of reversed polarity and the same magnetisation were used in the models at the locations indicated by arrows.



Anomalous delays of teleseismic P waves in Yellowstone National Park

TELESEISMIC P waves recorded by a short-period seismic network, comprising 12 stations, in Yellowstone National Park, show anomalous delays of 1-2 s in their travel times in the central region of the park relative to the surrounding area. To explain this phenomenon, I propose that a substantial body of low velocity material is present beneath the park, with horizontal dimensions of several tens of kilometres, it may be the magma chamber associated with the volcanism of Yellowstone (ref 1, and G P Eaton *et al*, unpublished).

This study is based on records of about 65 teleseisms, distributed in a narrow range of azimuths north, south-east, west-south-west, and north-west from Yellowstone. Distances to the events range from 25° to 90° . About 25 events are available from the south-east and north-west azimuths and 15 from the other two combined. Because of operational problems the number of events used in this study varies from station to station.

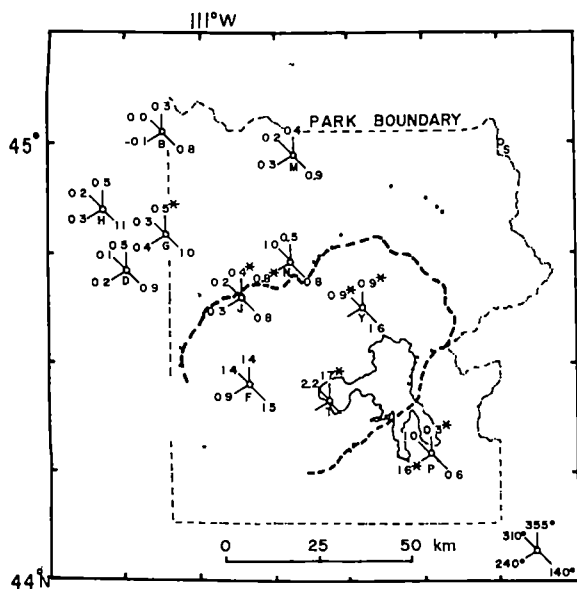


Fig. 1 Relative residuals in Yellowstone. Lettered dots, seismic stations; numbers, relative residuals with respect to station S, for events along the four azimuths (represented by the short lines); asterisks, uncertain values based on few available readings; dashed line, caldera boundary, inferred from geology and topography (ref 1, and G P Eaton, unpublished); dotted line, top of steep gravity gradient^{7,8}.

Travel-time residuals have been computed using the Seismological Tables for P by Herrin². These residuals contain a combination of station, path, and source effects and errors in earthquake location caused by the statistical nature of estimating hypocentres and origin times. Anomalous teleseismic residuals have been used in the past to study inhomogeneities in the physical properties of the Earth in the source regions of the earthquake³, along propagation paths of seismic waves⁴ and under recording stations^{5,6}. In order to isolate source and path effects from the effect of any anomalous structure in the immediate vicinity of the seismic network, one station is chosen as a reference. Residuals at the reference station are subtracted from the residuals at the other stations for each event and so-called relative residuals are obtained. Their variation across a network reflects the variation of the underlying crust and upper mantle.

The correct choice of reference station is critical, ideally it should be at a site which represents the average regional structure and which has no local anomalies. Geological evidence suggests that one of the stations well outside the Yellowstone caldera (ref 1, and G P Eaton *et al*, unpublished) is a good choice (Fig 1). To make this selection more rigorous, average residuals for events in the north-western and south-eastern azimuths have been computed at six stations at which more than 10 events are available for

each direction (Table 1). For the north-west, the residuals at the five stations outside the caldera are in the range 2.4–2.7 s but are about 1.5 s higher at station F (Fig 1), inside the caldera. For the south-eastern azimuth the residuals are 3.0–3.4 s at stations north-west of the caldera, and 4.0 s at station F. Thus, it seems that waves from the south-east, which pass under the Yellowstone caldera at some depth, are delayed relative to waves travelling from the north-west. Only at station S (Fig 1) is the average residual for both azimuths comparable to the residuals for waves that do not travel under the caldera. It therefore seems reasonable to assume that the average residuals at station S are representative of regional structure and independent of sub-caldera structures, so station S is used as the reference.

Relative residuals with respect to station S at four typical stations, for events in the north-westerly and south-easterly directions, plotted as a function of distance from the event, show clearly the azimuthally dependent travel-time delays in Yellowstone (Fig 2). At station F the relative residuals remain more or less constant at around 1.5 s for both azimuths, in the available distance range of 25°–85°, at stations G, M and B, on the other hand, the south-eastern residuals are consistently higher than the north-western values by about 0.8 s, suggesting the presence of a low velocity body under the caldera. There is some inevitable scatter in the plots, with a standard deviation of about 0.2 s around the average values. Of these stations, only station B shows a significant distance effect: the residuals around 80° are smaller than those at shorter distances by about 0.5 s. The angle of emergence of the rays changes from

Fig 2 Variation of relative residuals as a function of distance at stations F, G, M and B. Dots and crosses, events in the south-eastern and north-western azimuths, respectively; solid and dashed lines, average of the data for those directions.

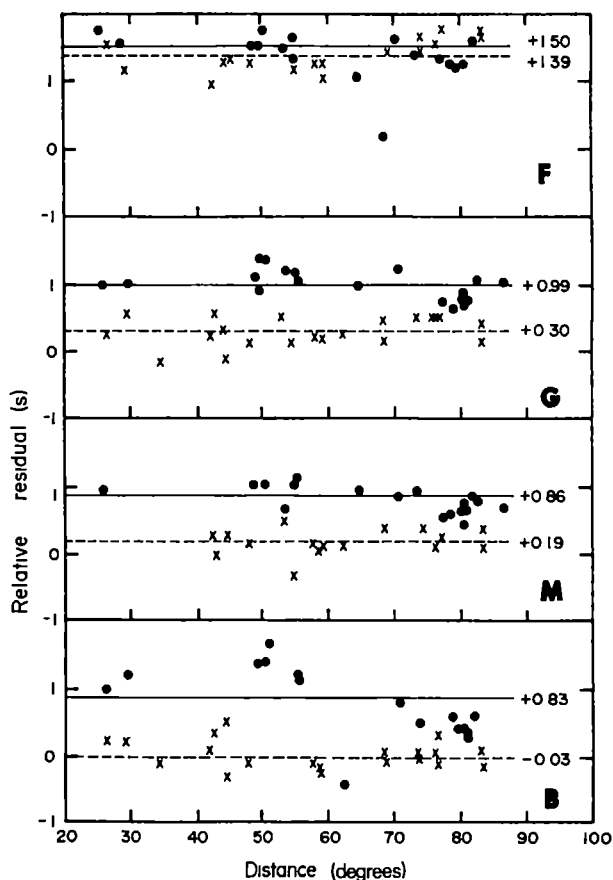


Table 1 Average residuals R , for events in the south-eastern and north-western azimuths

Station	South-east			North-west		
	No of events	R (s)	Standard deviation	No of events	R (s)	Standard deviation
M	23	3.3	0.6	17	2.7	0.5
F	19	4.0	0.5	19	3.9	0.4
G	22	3.4	0.6	23	2.7	0.5
D	14	3.0	0.6	17	2.7	0.5
B	18	3.2	0.7	23	2.4	0.5
S	22	2.3	0.6	23	2.5	0.6

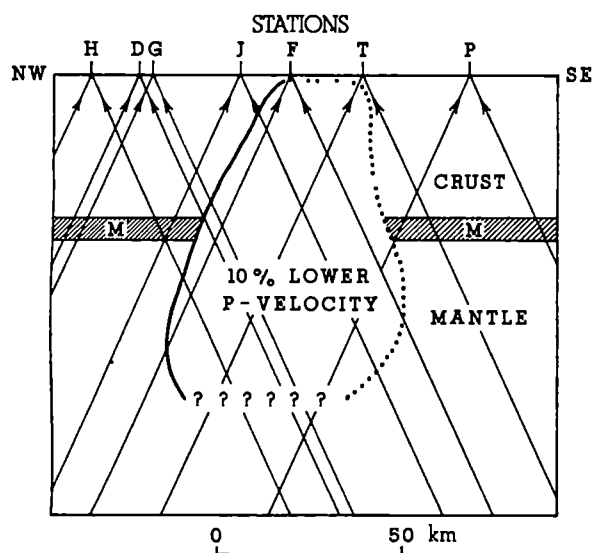


Fig 3 North-westerly trending vertical cross section of the low velocity body that possibly explains the travel time delay pattern. The boundary of the body is shown by a heavy solid line where well constrained by the data, by a dotted line where only poorly constrained, and by question marks where the boundary cannot be determined. Arrows, schematic ray paths for events in the north-western and south-eastern azimuths. Width of the dark shaded band (Moho) indicates uncertainty in crustal thickness¹³.

30° to 17° to the vertical as distance changes from 25° to 80°. The distance effect could occur if a low velocity body, of a particular shape and position provides equal path lengths for rays that reach stations F, G and M at various angles, while at station B, the steeper rays sample a deeper and probably narrower part of the body.

Consideration of the average relative residuals at stations F, Y and T within the caldera (Fig 1), shows that station F records large delays for all azimuths. Although the quality and quantity of data at stations Y and T are poor, the good data and a careful examination of all the available low quality seismograms show that stations Y and T are similar to station F, with high delays for all azimuths. Station N, at the edge of the caldera, almost fits into this pattern, except that it records a slightly lower northerly delay. As a first-order interpretation of the delay pattern at stations F, N, Y and T, it can be postulated a body with lower compressional wave velocity than the surrounding rocks exists under the central region of Yellowstone National Park. The top of the body must be close to the surface in the middle of the park, as shown by the large delays for all azimuths. At the north-western group of stations, M, B, G, H and D, which is outside the caldera, the relative residuals are about 1 s for events from the south-east and much lower for the other azimuths. The low velocity material must, therefore, extend to a depth such that the rays from the south-east reaching the north-western group of stations at angles of 17°–30° to the vertical traverse through that material.

It is also possible roughly to define the horizontal extent of the low velocity body. There is a sharp difference in the azimuthal pattern of relative residuals between stations F and J (Fig 2). The delays are high for all azimuths at station F, whereas at station J, the south-easterly delay is much higher than for the other azimuths, indicating the existence of a boundary to the body to the south-east at some depth from station J. The northerly delays decrease from the high values at stations F, T and Y to about 0.5 s

at stations J and N, indicating shorter paths through the body for rays reaching the latter stations, that indicates a boundary not far north of the caldera boundary. South-easterly delays seem to decrease between stations Y and P, placing a limit on the south-easterly extent of the body. The available data are not inconsistent with the *ad hoc* hypothesis that the horizontal boundary of the top of the body is near the caldera rim.

The region of the Yellowstone caldera has a negative Bouguer gravity anomaly with sharp gradients close to the geologically determined caldera boundary^{7,8} (Fig 1). The gravity low is believed to be produced by the partial presence of low density material such as rhyolite flows, magma and caldera fill. That explains only a small fraction of any observed teleseismic delays, however. A 10 km thickness of material with a conducting seismic velocity 10% lower than the surrounding rock would introduce a delay of only 0.2 s. Only teleseismic evidence, in particular the large delays at the north-western group of stations, shows that seismic rays are delayed at some depth under the caldera. High frequency seismic waves from local earthquakes in the region are severely attenuated as they cross the Yellowstone caldera (G. P. Eaton *et al.*, unpublished). Yellowstone has a history of volcanism extending from about 2 Myr ago to as recently as 0.07 Myr ago. A low velocity body comprising rhyolitic magma a few kilometres thick overlying an immense volume of basaltic magma and thermally disturbed crustal and mantle rocks, could be responsible for the gravity anomaly, the seismic wave attenuation, and the teleseismic delays, it is also consistent with the geological evidence (ref 1, and G. P. Eaton, unpublished).

A tentative model for the low velocity body is suggested. If the compressional velocity in this body is assumed to be 10% lower than in the surrounding granite, the pattern of delays observed along a section from stations P to H are explicable (Fig 3). This is a very conservative model based on an angle of emergence at the surface of 25°, for events at an average distance of about 50° from Yellowstone.

It has been proposed that Yellowstone lies above a deep mantle 'plume' of hot magma, perhaps 100–200 km in diameter, rising from the lower mantle^{10,11}. Anomalous seismic velocities, recorded using teleseismic methods, have been reported near the core-mantle boundary under Hawaii, another likely 'plume' region^{4,12,13}. It is of interest that even at the most north-western station, B, large south-easterly delays still persist, indicating that the anomalous body is perhaps deeper than the maximum depth seen by the network. Preliminary results, obtained using a north-westerly profile of portable stations extending about 200 km from the boundary of Yellowstone Park, show that the anomalous body may extend several hundred kilometres deep into the upper mantle¹⁴.

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THE Kuroko strata-bound sulphide deposits in Japan, which are mined for lead, zinc, copper, silver and gold, are apparently related to Middle Miocene submarine acidic volcanism of the Green Tuff region¹⁻⁵ (Fig. 1). Most of the Kuroko deposits occur in the upper part of the Nishikurosawa stage (≈ 14 Myr) of the Middle Miocene and, on geological grounds, are considered to have been formed in a short time⁶. Here I describe an attempt to determine the length of time involved from the magnetic polarity of associated rocks.

Most of the results have been obtained from red ferruginous chert, which commonly overlies the Koroko ore in beds 1–10 m thick. Chert deposition seems to have been the last stage in the Kuroko mineralisation process⁷. The chert consists of quartz (diameter 10–600 μm) and very fine grained haematite, with minor pyrite and barite. The average grain sizes^{8,9} of the haematite determined from Furutobe and Kosaka mines (Fig. 1) are 150 \AA and 95 \AA respectively. The initial grain size between the single-domain and superparamagnetic range is usually assumed to be about 290 \AA (ref. 10), so much of the haematite is probably superparamagnetic. Nevertheless a substantial number of larger grains must be present because the remanent magnetisation is very stable. The remanent intensity drops only a few per cent, and the directions remain unchanged, after demagnetisation in alternating fields of 600 oersted. Also hysteresis loops for fields up to 19,000 oersted show essentially a straight-line increase, with no tendency to saturation. The magnetisation is probably a chemical remanent magnetisation^{11,12}.

Fig. 1 Distribution of Kuroko deposits *a*, Zone of Miocene volcanism, *b*, zone of Neogene folding

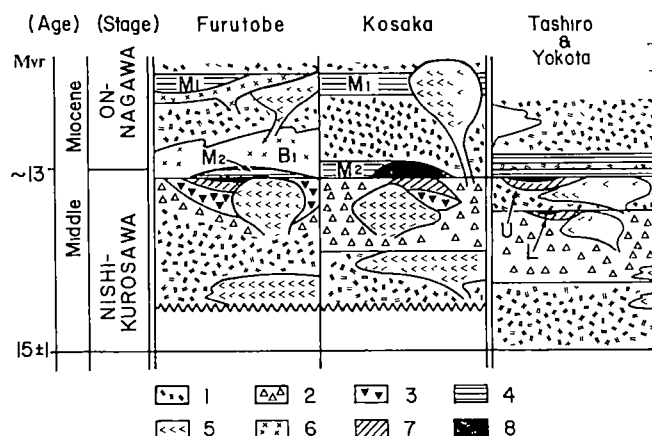
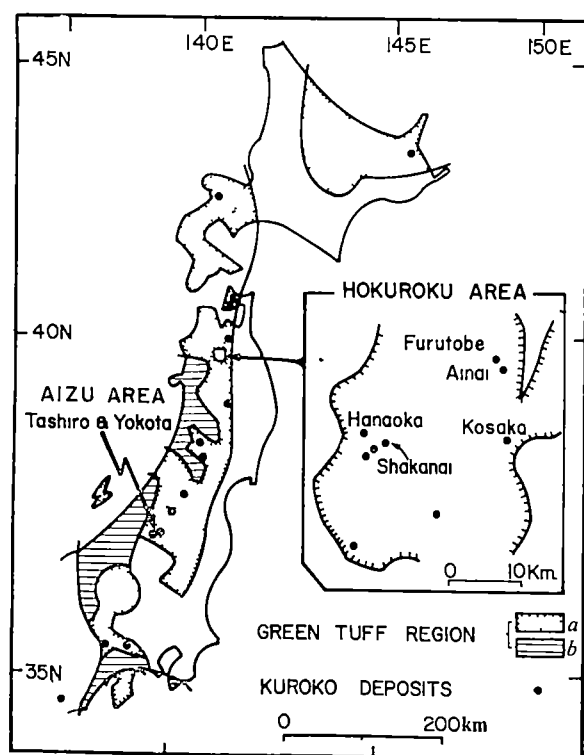


Fig. 2 Geological columns of the Furutoba and Kosaka mining districts in the Hokuroku area, and of the Tashiro and Yokota mining districts in the Aizu area. 1, Tuff, 2, tuff breccia, 3, volcanic breccia, 4, mudstone, 5, rhyolite, 6, basalt, 7, Kuroko ore body, 8, ferruginous chert. L, Lower ore bodies, U, Upper ore bodies. For explanation of M₁, M₂ and B see text. The ages are from Ikebe *et al.*²¹

(Fig. 2) The lower half of M_2 has microfossils of the Nishikurosawa stage and its upper half has microfossils of the On-nagawa stage^{3,13}. At Furutobe mine in the Hokuroku area the sequence is Kuroko ore, ferruginous chert, mudstone M_2 and basalt B_1 (ref. 14) (Fig. 3). The chert has reversed magnetisation (10 samples, mean intensity 500 μ gauss), the mudstone has normal magnetisation (three samples, intensity 50 μ gauss, remanent coercivity 200 oersted) and the basalt has normal magnetisation (three samples, intensity 2,000 μ gauss, remanent coercivity 150 oersted). At Aina mine¹⁵ the ferruginous chert has reversed magnetisation (five samples, intensity 50 μ gauss). The chert at Hanaoka mine has both normal and reversed magnetisation (two normal samples, six reversed samples, intensity 30 μ gauss) and at Shakanai mine⁵ the ferruginous chert that occurs in the lower part of the ore body has reversed magnetisation (three samples, intensity 200 μ gauss). The ferruginous chert at Kosaka mine occurs in the mudstone M_2 (ref. 16) (Fig. 3). Samples taken from directly above, and at some distance from, Kuroko ore have normal magnetisation (nine samples, intensity 100–800 μ gauss).

The Tashiro and Yokota mines in the Aizu area have similar geological sequences¹⁷. There are two mudstone layers, the lower contains microfossils of the Nishikurosawa stage, and the upper microfossils of the On-nagawa stage, just as in the Hokuroku area (Fig. 2). The Aizu area is exceptional in that there are two different ore horizons—there is an upper ore body at the same horizon as in the Hokuroku area and a lower one (Fig. 2). Two layers of chert directly overlay both upper and lower ore bodies. Ferruginous chert associated with the upper ore bodies has reversed magnetisation (six samples, intensity 50 μ gauss) but that associated with the lower ore bodies has normal magnetisation (six samples, 50 μ gauss).

The polarity results are plotted on the basis of the known stratigraphy in Fig. 3. All the Kuroko deposits, except the lower ore bodies in the Aizu area, seem to have been formed in one reversed polarity interval. Soon after the formation of the Kuroko deposits the geomagnetic field changed from reversed to normal, and lower ore bodies in the Aizu area were apparently formed in the preceding normal interval. The upper part of the Nishikurosawa stage seems, however, to have been laid down in an interval of reversed polarity. This is consistent with evidence of the short reversal interval in the upper part of the Nishikurosawa stage from measurements on volcanic and sedimentary rocks in the Green Tuff region^{18,19}

If the magnetic stratigraphy of the Middle Miocene were perfectly known, the exact time interval during which the

Kuroko deposits formed could be determined from the evidence summarised in Fig 3. Of course this is not yet possible, but an estimate can be made from the time scale of Heirtzler *et al.*²⁰. On this scale five reversed intervals occur between 12 and 15 Myr. As the Kuroko deposits, apart from a few exceptional deposits which occur in a different horizon, were formed in a single polarity interval within that time span, it is reasonable to conclude that they were laid down in less than 0.2 Myr. These results support the supposition that the Kuroko ores are of syngenetic origin¹⁻⁷. They also indicate that the ores were deposited essentially contemporaneously in both the Aizu and Hokuroku areas, more than 300 km apart.

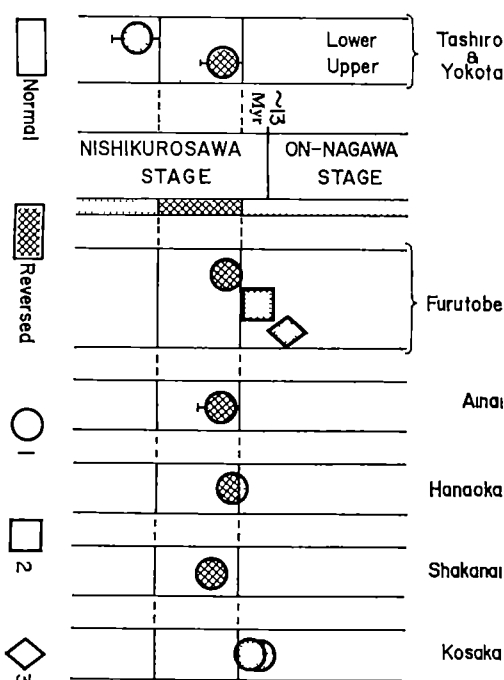


Fig. 3 Polarities plotted on the basis of the known stratigraphic sequence 1, Ferruginous chert, 2, mudstone, 3, basalt. Upper and Lower are the upper and lower ore bodies in the Aizu area.

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Deglaciation of the Labrador continental shelf

It has long been inferred from studies of glacial features on land that the North American Quaternary glaciers extended far out on the Atlantic continental shelf^{1,2}. Only within the last ten years, however, have offshore studies^{3,4} on the Scotian Shelf convincingly demonstrated the presence of a system of submarine moraines. This study, which used 1,100 km of deep-towed 3.5 kHz sparker profiles, 1,200 km of side-scan sonar records, 2,000 km of echo sounding profiles and 165 bottom sediment samples collected during cruises of CSS Dawson and CFAV Sackville has identified moraines and other relict glacial land forms on the outer Labrador Shelf.

More than 20% of the Hamilton Bank area is topographically rough (bottom type I, Fig. 1) and has a local relief of 10-30 m (Fig. 2c, d). Side-scan sonar representations of this irregular surface are characterised by mottles, bathymetric charts⁵ show numerous roughly circular, closed depressions deeper than 10 m. Adjacent areas with a more gently undulating surface without closed bathymetric depressions are recognised as a separate morphological region (bottom type II, Fig. 1). A zone of extremely smooth, flat topography (bottom type III, Fig. 1) is distinguished as still another morphological unit. Side-scan records reveal that the uniform bottom in this area is relieved only by occasional iceberg plow marks. Sparker profiles reveal a hard bottom with acoustic penetration generally <10 m, similar to the rest of the bank.

Moraines of the Hamilton Bank region (Fig. 2a, b) seem identical in dimensions and in transverse section to those of the Scotian Shelf³ and to terrestrial end moraines in southern New England⁶. Moraines are traceable along the foot of the landward facing bank margin and across the surface of the bank (Fig. 1).

The sharpness of existing relict features implies that they have been affected little by post-glacial sedimentary and erosional processes. Surface sediments, however, are not relict and seem to have reached a partial equilibrium with present oceanographic conditions. In general, the sediments are similar to those reported from elsewhere in glaciated offshore eastern Canada and New England^{3,6-10}.

Identification of probable end moraines and the ubiquitous distribution of coarse gravel and cobbles, as well as the excellent state of preservation of glacial landforms, strongly imply that the entire continental shelf was glaciated by the Laurentide ice sheet during the Wisconsinan. Coarse sediments, which appear in places on the continental slope to at least a depth of 400 m, may have been deposited beneath a floating ice shelf¹¹. Sills covered by a coarse gravel and boulder lag at the seaward end of Cartwright and Hawke Channels are interpreted to be mounds of detritus deposited beneath the ice shelf at a point just seaward of the grounding line¹². Although Hamilton Bank was completely ice covered, the greatest ice discharge seems to have been through these transverse channels. There is on average about 100-150 m of glacial drift in the channels compared with 50 m on the bank^{13,14}.

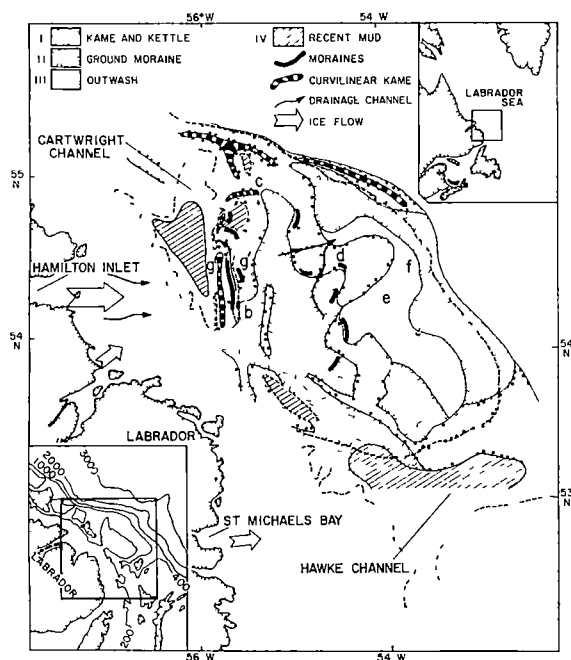


Fig 1 Physiographic-surficial geology map of the Hamilton Bank area. The dashed line is the 300 m contour and all areas completely enclosed by this contour are basins. Letters refer to sections in Fig 2. Flow directions of continental ice are inferred from the Glacial Map of Canada²⁰.

Once deglaciation had begun, ice on the bank must have retreated by calving until an equilibrium between ice thickness and water depth was reached^{15,16}. Bottom type I (Fig 1) seems to have formed as the remaining portion of the grounded ice sheet melted *in situ*. Morphologically, this bottom closely resembles terrestrial kame and kettle topography. This, together with the stratification detected in cores¹⁰, leads me to term these features 'subaqueous' kames and kettles. Kames probably formed beneath the stranded, stagnant ice sheet (Fig 3) as cavities caused by uneven bottom melting filled with an accumulation of semi-stratified detritus dropped from the undersurface of the ice¹¹. Subaqueous kettle holes seem to be the result of the disintegration and flotation of grounded masses of ice that had been surrounded by drift.

Melt water flow and perhaps tidal currents beneath the grounded ice formed a system of drainage channels (Fig 1) and provided a route for the transport of bed load and suspended sediment by hyperpycnal flow to deglaciated portions of the shelf. The resulting deposits, winnowed and spread southward over previously deposited drift by the prevailing Labrador Current, may have created bottom type III (Fig 1). This area is as flat as (Fig 2e, f), and seems to be analogous to, terrestrial outwash plains such as those of New England on Martha's Vineyard and Long Island².

Curvilinear kame features (Fig 1) are recognised by their relatively smooth flat tops and weak stratification with some evidence of ice contact disruption of bedding near the margins (Fig 2g-g'). They are thought to have formed where debris laden glacial runoff was impounded in temporary ice lakes or bays situated between ice tongues. The transverse channel sills and a terrace along the seaward edge of the bank (Fig 1), although generated beneath a floating ice shelf, are included in this category because it seems probable that they consist of semi-stratified drift. In contrast, end moraines on the bank are believed to have formed along a grounded ice sheet margin. Their irregular topography is seen as indicative of rapid deposition with little chance for the development of stratification. The

curvilinear trend of end moraines across the central bank (Fig 1) was probably formed during a readvance of the bank ice cover following the initiation of general deglaciation. The gently undulating surface (bottom type II, Fig 1) which may be ground moraine, marks the area overridden by this readvance.

Preserved ground moraine and a lack of subaqueous kames and kettles landward of the end moraine complex indicate that once the readvance had ceased, the ice sheet broke up and dispersed with little melting in place. For the remainder of the deglaciation period, active ice tongues and stagnant ice lobes were confined to the transverse channels where they gradually decreased in size leaving a complex pattern of marginal and end moraines, kame terraces and kame and kettle topography.

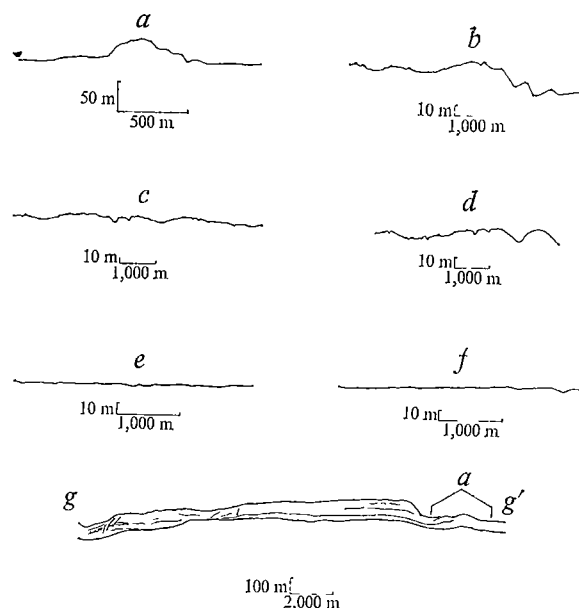


Fig 2 Traced profiles of glacial and pro-glacial features on Hamilton Bank. a, b, moraines; c, d, kame and kettle topography; e, f, outwash plain; g-g', large linear kame, with weak stratification evident. Types of profiles include echo sounding (a), deep-towed sparker (b, c, d, e, f) and 5 inch³ air gun interpretation (g-g', ref. 29).

It is significant that Wisconsinan glacial ice was grounded on Hamilton Bank and in the transverse channels, because it has been established that at water depths greater than a critical value, buoyancy and the strength of glacial ice permit the survival of only those ice sheets that have sufficient lateral resistance to spreading¹⁵⁻¹⁸. This resistance may be supplied by confinement between anchoring headlines or by a strong coupling to the substrate as in a cold (frozen) based ice sheet¹⁵. In West Antarctica the critical depth is about 170 m (ref. 17). Allowing for a Wisconsin glacioeustatic sea level lowering of 100 m (ref. 19), 80 m or more of glacioisostatic depression on the bank, which today averages about 190 m below sea level, would have produced depths greater than 170 m. Because the levels of raised beaches and marine deposits along the south-eastern Labrador coast²⁰ make at least that amount of glacioisostatic depression extremely likely, an initially cold based ice sheet on the bank is strongly implied. Thus, deglaciation of the Labrador Shelf in the Hamilton Bank area may have been directly caused by a decreased resistance to spreading by decoupling the ice from the substrate. This occurred

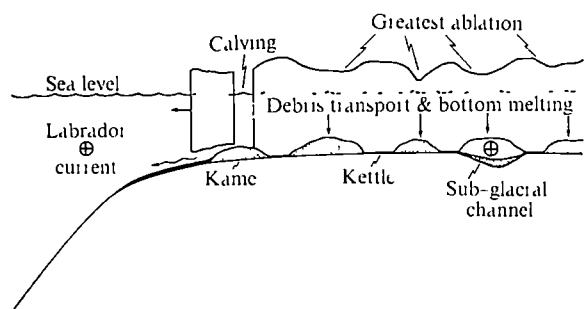


Fig. 3 Schematic representation of hummocky stagnant ice sheet on Hamilton Bank. Stippling represents semi-stratified drift and channel deposits. Black shading is outwash transported by hyperpycnal outflow (wavy arrow) from subglacial channels. This may have been augmented by tidal currents. Debris freed from the ice sheet by bottom melting is shown accumulating on ice-free portions of the bottom forming subaqueous kame deposits. Subaqueous kettles are produced by the flotation and removal of grounded ice masses.

probably during a period of rising summer air temperatures which warmed the ice sheet and precipitated a change from cold to wet-based glacial conditions. In Antarctica grounded ice sheets and ice shelves are thought to be unstable where the average air temperature for the warmest month is above 0°C (ref. 16).

Faunal evidence indicates a warming of ocean water in the eastern Arctic region beginning before 8,000 yr BP (refs 21, 22). The Cockburn-Cockrane readvance of closely similar age has been interpreted²⁴⁻²⁵ to be the result of a glacial surge to a new equilibrium profile in response to rapid deglaciation of a part of Hudson Bay²³. It is therefore tempting to speculate that the retreat of ice from the seaward edge of Hamilton Bank occurred at about the same time as the opening of Hudson Bay and that both areas were deglaciated in response to a general warming of the ice sheet. The ensuing readvance on Hamilton Bank is seen as an adjustment to a new equilibrium profile and as a corollary to the Cockburn-Cockrane event²³.

A radiometric deglacial chronology for south-eastern Labrador has not yet been established. Deglaciation dates of 16,000–14,000 yr BP have been inferred for the Labrador Shelf²³, but published²⁶ and unpublished ¹⁴C dates from the Hamlet Inlet area are consistently younger than 8,700 yr BP. These dates and the probable triggering effect of the climatic warming imply that deglaciation of the Labrador Shelf may have been in progress as late as 9,000 yr BP (refs 26–28).

Eastcan Exploration Ltd, Calgary, supplied the V-fin used in this study. Information on unpublished ¹⁴C dates was supplied by R. J. Fulton of the Geological Survey of Canada. Dr W. J. M. van der Linden proposed this project and collected most of the data.

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Survey of three megalithic sites in Argyllshire

THREE sites showing simple alignments of standing stones in Argyllshire were tested for possible astronomical significance by members of the Cambridge University Astronomical Society during August and September 1973. The sites were surveyed in detail with a portable (20" circles) vernier theodolite, on which comprehensive tests were made before and after the expedition to determine adjustment errors, brief checks were made on site. Absolute azimuths were determined accurately by making timed observations of the Sun using MSF Rugby as the standard time signal. The error in azimuth was normally $\pm 10''$ and in altitude $\pm 20''$, although the latter value had to be modified to take account of uncertainties in atmospheric refraction. There is extensive information available on the Ballochroy site¹⁻³, and brief details of the sites at Loch Seil⁴⁻⁶ and Loch Nell^{4,6} have been published.

As so much importance has been attached to the site at Ballochroy we surveyed it hoping to produce more accurate profile measurements. The site contains three menhirs aligned with the Island of Cara to the south-west, with the flattened faces of the central stone indicating approximately the direction of Corra Bheinn on Jura to the north-west. A kist lies 40 m south-west of the three menhirs, and is

Fig. 1 Midwinter sunset over Cara Island (NR638438) shown for, a, 1600 BC (obliquity of ecliptic, $\epsilon = 23^{\circ}53'09''$) and, b, 1800 BC ($\epsilon = 23^{\circ}54'28''$) as seen from the menhirs or the kist at Ballochroy. Profile of Cara shown below. Errors in altitude of $\pm 0.7'$ are estimated from a temperature variation of $\pm 3^{\circ}\text{C}$ around 5°C at sunset, and from a measurement error of $\pm 0.35'$.

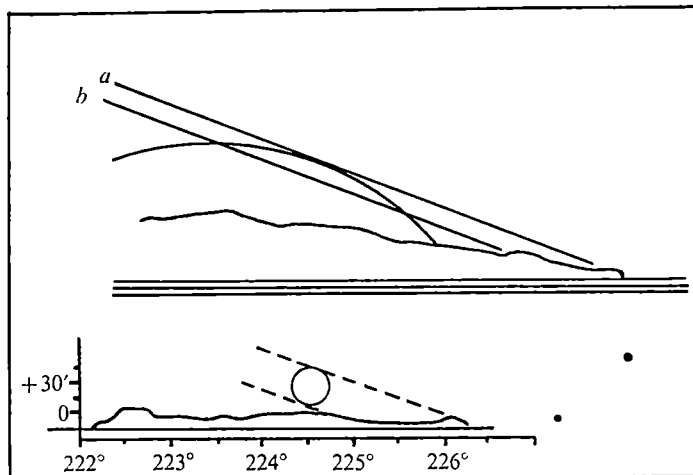


Table 1 Observed lines at Loch Seil (*a, b, c*) and Strontouiller (*d, e, f*)

Line	Az*	h*	δ^*	Notes
<i>a</i>			-21°	Alignment—no obvious foresight
<i>b</i>	326.2°	+5.3°	+32.1°	Indicated foresight
<i>c</i>			+6°	Alignment—no obvious foresight
<i>d</i>	305.3°	+3.6°	+20.9°	Cross sight line, notch
<i>e</i>	307.5°	+3.0°	+21.5°	Cross sight line, pit
<i>f</i>			-21.8°	Ref 3 (not surveyed—trees)

*Azimuth (Az), altitude (h) and declination (δ) are given for well defined lines

approximately collinear with them. Weather conditions during our survey were variable, especially over the Paps of Jura, but repeated observations over a 3-h period showed refraction to be constant. For calculating the track of the setting Sun we used astronomical refraction tables^{7,8}. We followed Thom's method⁹ to allow for changes in our measured altitudes caused by local terrestrial refraction.

An observer at the site looking towards Cara in 1580 (± 100) BC would have seen the top limb of the midwinter Sun set just clear of the island (Fig 1). An observer looking towards the north-west from the central stone would have seen the midsummer Sun's upper limb clip the top of Corra Bheinn in 1640 (± 70) BC, standing near the kist in 1600 (± 70) BC he would have seen the Sun twinkle down the slope of Corra Bheinn (Fig 2). Thom postulated^{1,3} that the site was constructed to observe these phenomena. The accuracy of our survey and the similarity between the dates quoted lends support to this view.

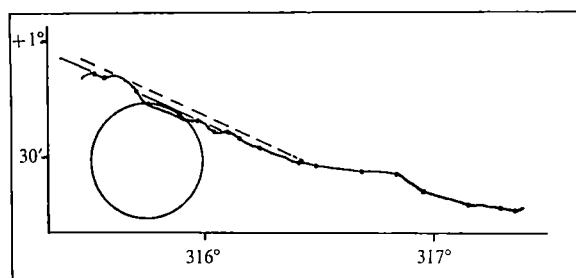


Fig 2 Midsummer sunset behind Corra Bheinn (NR526755) at 1600 BC ($\epsilon = 23^\circ 53' 09''$) as seen from centre menhir (---) and kist (—). Errors in profile altitudes of $\pm 0.5'$ are estimated from a temperature variation of $\pm 3^\circ\text{C}$ around 13°C at sunset, and from a measurement error of $\pm 0.35'$.

The principal stones on the site near Loch Seil, Kilmiver are three menhirs 2.5 m high, the centre stone is somewhat tilted. The stump of another menhir, apparently broken since Thom's survey³, was found 40 m away. The possible stump of another menhir, accurately in line with the other outlier and the centre menhir of the group of three, was found 180 m away. Skyline profiles, all of which were less than 3 km distant, were surveyed in both directions along the line of the three principal stones (Table 1, *a* and *b*), and in the direction indicated by the outliers (Table 1, *c*). Declinations for these lines are given in Table 1, we agree with Thom's declinations⁴ but find no well indicated foresight for his solar line and do not think his Capella line significant.

The site at Loch Nell, near Oban, is near a farm the name of which—Strontouiller—is Gaelic for 'point of light'. It has a menhir 4 m high and a possible 1 m menhir 50 m to the south-east with a ring cairn 3 m in diameter between them. The small menhir has a gate hung from it, but because of its size and shape it seems unlikely that it

was erected for this purpose. About 300 m to the north-west of the large menhir is a circle (15 m in diameter) of boulders, which is possibly all that remains of a despoiled cairn. An outcrop of rock, on which there seems to be a cairn 20 m in diameter, lies between the circle and the menhirs and restricts the view of the menhirs from the centre of the circle. The two menhirs are aligned with a small hill 600 m away.

The top of the larger menhir has been flattened (an unusual feature) and slopes at the same angle as the hill. The cross-sight lines indicated by opposite edges of the menhirs point accurately at two prominent gaps on the hillside (Fig 3). One is a pit 4.5 m wide which has earthworks at its sides. The other is an apparently artificial, 1 m wide, V-shaped notch cut into the rock. The notch and pit

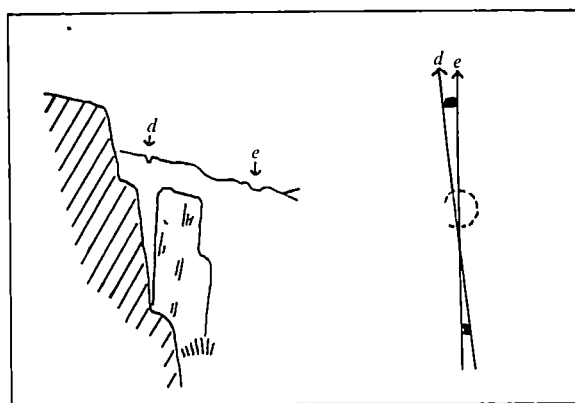


Fig 3 Left, skyline profile to the west at Strontouiller seen from behind the 1 m menhir, 4 m menhir indicates notch *d*. Right, cross bearings indicate notches *d* and *e* with setting declinations $20^\circ 56' 1''$ and $21^\circ 28' 2''$. Ring cairn shown dotted.

lines (Table 1, *d* and *e*) indicate accurately a limb of the setting Sun at declinations $20^\circ 56' 1''$ and $21^\circ 28' 2''$ respectively. We think that these declinations have no particular astronomical significance. Thom⁴ gives another (calendar) line at this site (Table 1, *f*), indicated by the large menhir from the circle. The Ordnance Survey marks the farm on the hill as 'Duneil' (dun is Gaelic for fort), which suggests that the earthworks are of recent rather than megalithic origin. Also, there are further earthworks on the southern slope and behind the hill. Thus, we conclude that such convincing alignments may have occurred only by chance, this view, however, requires confirmation by independent archaeological work. (Other profiles indicated by the flat sides of the large menhir were surveyed but these too, in our opinion, have no astronomical significance.)

The survey of Ballochroy shows that the site could have been used to observe the midwinter and midsummer setting Sun in 1600 BC. The alignments at the other two sites are not, in our opinion, consistent with possible astronomical usage, despite the existence at the Loch Nell site of seemingly convincing artificial foresights.

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Mineralogical alteration of Chinese tomb jades

ADMIRERS of ancient Chinese jades have frequently noted the curious white, chalky areas which invade the otherwise firm polished surfaces of many artefacts. This phenomenon, variously described as "alteration", "calcification", "calcination", or simply "decay", seems to be restricted to ancient (Shang to Han Dynasty, 1766 BC to 220 AD) 'tomb jades', artefacts explicitly or implicitly connected with burials¹. We have examined artefacts exhibiting this alteration using X-ray powder diffraction and scanning electron microscopy. We postulate that the alteration consists of a selective dissolution (leaching) along grain boundaries by ammoniacal solutions of high pH produced during decay of the corpses with which the jades were buried. Analyses by

Fig. 1 Chou Dynasty tiger (?) (Buffalo Museum of Science No. J-294) of pale green nephrite, illustrating typical partial alteration. Overall length 5.3 cm.

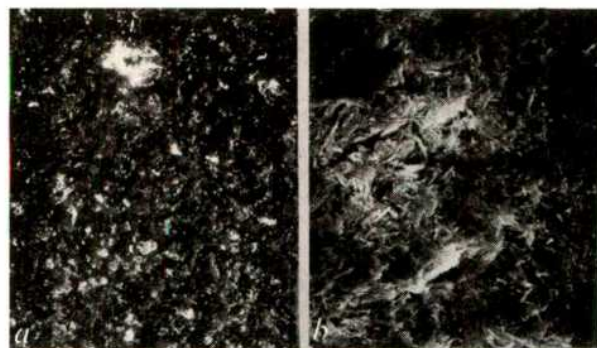


Fig. 2 Scanning electron photomicrographs of surface of nephrite artefact. *a*, Unaltered surface (width of field 100 μ m); *b*, altered surface (width of field 100 μ m).

X-ray powder diffraction of a number of partially altered jades showed them all to be nephrite (a fine grained, compact variety of the tremolite-actinolite series of amphiboles). This mineralogy is consistent with the antiquity of the artefacts, since the other jade mineral, jadeite (a pyroxene), was not introduced into China until the eighteenth century AD^{1,2}. Interestingly, the diffraction patterns of altered and unaltered regions are identical (also noted by West³), indicating that the alteration does not involve any change in mineral composition. This invalidates the terms calcification and calcination which, respectively, imply chemical replacement and thermal decomposition. It is likely that these terms were coined with reference to the white, ivory-like 'chicken-bone' jades which are produced by heating nephrite: calcification because of the bone-like appearance, and calcination referring to the method of production. The original nephrite in the 'chicken-bone' jades has, however, decomposed with loss of structural water into a diopside-enstatite-silica assemblage which is easily distinguished in diffraction patterns.

Altered surfaces are characteristically white or buff-coloured; they are softer and less reflective than adjacent unaltered areas. Occasionally artefacts will be completely altered, usually with badly eroded surfaces, although more commonly, only certain areas are altered (Fig. 1), with only shallow penetration, preserving the details of intricately carved surfaces. The boundaries between altered and unaltered regions are typically relatively sharp. A curious patterned alteration occurs on some Archaic ceremonial knife blades, described as "fabric-marked", in the form of small orientated blebs, reminiscent of the pattern of a coarsely woven cloth.

The change in colour, hardness, and reflectivity of the altered surface, coupled with the lack of change in mineralogy, suggests chemical etching as a likely cause of the alteration. We therefore examined the surface microstructure of altered and unaltered areas of a Chou Dynasty (1122 to 206 BC) artefact (Fig. 1) with a scanning electron microscope. The results show that the unaltered surface is compact and smooth, even at high magnification (Fig. 2*a*), whereas the altered surface consists of an open, felted network of acicular tremolite crystals (Fig. 2*b*). (Comparative X-ray emission spectroscopy of altered and unaltered surfaces reveals no significant difference in chemical composition.)

This suggests that the mechanism of alteration is a selective congruent dissolution (leaching) along grain boundaries. It has previously been suggested that the alteration may result from the action of soil acids and moisture^{1,2}, and may be accelerated by decomposition products of the bodies in the tombs¹. Experimental studies³ indicate that the solubility of tremolite is at a minimum in solutions of pH from 3 to

8, the range of most natural soil waters. Furthermore, solubility studies of tremolite and other silicate minerals show that dissolution in acidic waters is incongruent, leaving a solid residue of silica, and is extremely slow⁴. Dissolution in basic ($pH \geq 9$) waters, however, is congruent and relatively rapid.

There seems to be no correlation between the degree of alteration of nephrite artefacts and their archaeological age or their length of burial. Thus chemical 'weathering', resulting from the action of soil moisture, does not seem to be a viable explanation for the observed alteration. We propose that the alteration consists of congruent dissolution of nephrite, preferentially along grain boundaries, that the alteration takes place in relatively short times, perhaps months, and that it is produced by the action of basic (high pH) rather than acidic solutions. Alteration of select areas of nephrite in sharp contact with unaltered areas may result from differences in the fabric (microstructure) of domains in the polycrystalline material⁵.

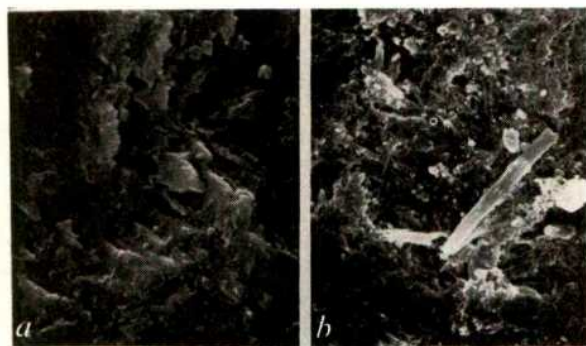


Fig. 3 Scanning electron photomicrographs of *a*, artificially altered nephrite surface (width of field 10 μm); and *b*, altered surface of nephrite artefact (width of field 33 μm).

Conditions suitable for such alteration could be produced and maintained for adequate periods in the tomb environment. Degradation of proteins during liquification of body tissues accompanied by bacterial decay could produce ammoniacal solutions of high pH . In a sealed coffin, such solutions would alter nephrite artefacts buried with the body. The degree of alteration would thus be a function of the degree and duration of contact between the artefact and solution as well as the microfabric of the nephrite. Fabric-marked blades could result from 'wicking' of basic solutions to the blade surface by cloth funerary wrappings.

To test this hypothesis we immersed polished nephrite slabs in solutions containing ammonium hydroxide for several weeks at room temperature. The resulting alteration (Fig. 3*a*) is essentially identical to, although less severe than, that of the artefact examined (Fig. 3*b*). The partial alteration of this nephrite, which was totally immersed in the solution, illustrates the importance of the microfabric to the rate of alteration. Domains of randomly oriented tremolite needles are more susceptible to dissolution than those possessing a high degree of orientation. Alteration also occurred more rapidly along fractures and unpolished surfaces, presumably as a result of disruption of oriented microfabrics at fracture surfaces.

The ancient Chinese buried jade with their dead to prevent decomposition of the body—during the Han Dynasty pieces of jade were placed in each of the body orifices to ward off decay. It is ironic that the corpses caused the decay of the jade.

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Adsorption of divalent phosphate on hydrous aluminium oxide

THE adsorption of phosphate on aluminium and iron hydrous oxides is of practical importance in soils because of its influence on the availability of this nutrient to plants. One aspect of phosphate adsorption is the release of hydroxyl ions into the solution. This paper reports a quantitative relationship between divalent phosphate adsorbed on, and hydroxyl ions released from, hydrous alumina at different phosphate concentrations. From the relationship it is proposed that the phosphate ions are bonded to aluminium atoms forming both monodentate and bridged complexes.

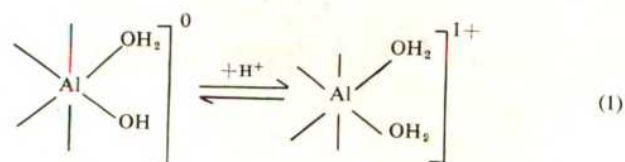
The preparation and properties of the aluminium oxide used have been described elsewhere¹. The sample was predominantly amorphous and had a surface area (B.E.T.) of 197 $m^2 g^{-1}$. The point of zero charge (PZC) pH (ref. 2) was 9.3. The phosphate adsorption and hydroxyl ion release were determined using an automatic titrator³. The adsorption was carried out for 3 h at pH 8.5 in 10 $\mu mol cm^{-3}$ KCl at 30° C under a nitrogen atmosphere. The high pH was chosen so that phosphate in solution existed as divalent ions.

The phosphate adsorption did not reach a maximum (Fig. 1). The theoretical isotherm obtained by using a two-term Langmuir's equation³ was in agreement with the observed adsorption values up to about 280 $\mu mol g^{-1}$, suggesting that at higher values phosphate was adsorbed by different types of sites.

The hydroxyl ions released plotted against phosphate adsorbed (Fig. 2) yielded two straight lines. The ratio of hydroxyl ions released to phosphate adsorbed, R , was 1.44 and 1.07 at less than and greater than 212 μmol of phosphate adsorbed, respectively.

To elucidate the probable mechanism of phosphate adsorption, both the charge characteristic of the oxide solution interface and the phosphate species in solution should be taken into account.

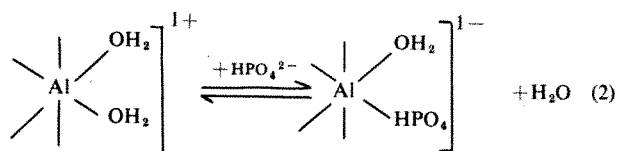
The sign and the magnitude of surface charge on the interface is determined by H^+ and OH^- ions in the absence of other specifically adsorbed anions². At the acid side of the PZC pH the oxide surface can be considered to consist of neutral and positive sites (1).



In the present sample only 40 $\mu mol g^{-1}$ of protons were adsorbed in decreasing the pH from 9.3 (PZC pH) to 8.5.

Considering the great amount of phosphate adsorbed by comparison with the net positive charge, and also the amount of hydroxyl ions released, most of the phosphate was probably adsorbed on neutral sites.

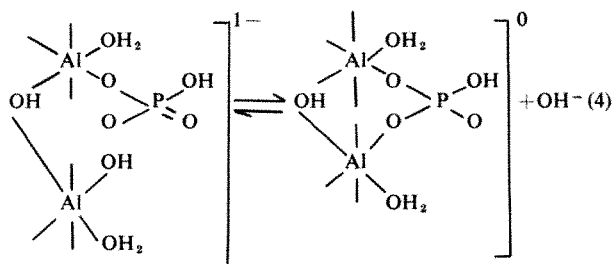
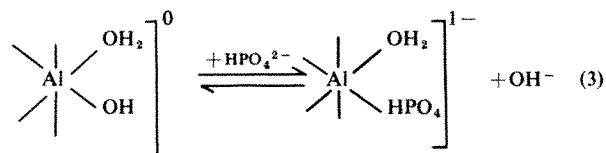
The following ligand exchange reactions can be envisaged for adsorption of phosphate at $< 212 \mu\text{mol}$:



For the reaction as in equation (2) $R = 0$ and for that in equation (3) $R = 1$.

It is probable that the ligand exchange on the neutral adsorption sites is mediated by protons made available by dissociation of the monohydrogen phosphate at the oxide surface⁴. The dissociated form, however, seems to have a transitory existence, for the evidence is that the adsorbed phosphate rapidly acquires a proton from the solvent and equilibrates with the species in solution¹.

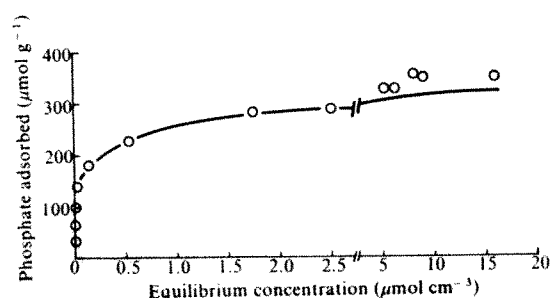
The exchange reactions suggested above do not, however, account for R being > 1 . One possible reaction is the formation of binuclear aluminium phosphate complexes with phosphate bridging across unshared corners of adjacent aluminium atoms (4) following the adsorption as in equation (3).



The value of R for the combined reactions of equations (3) and (4) is 2.

Similar models for the presence of a bridging ligand configuration of the adsorbed phosphate have been proposed from phosphate desorption⁵, kinetics of isotopic exchange⁶ and change in surface charge studies⁷. The R value of 1.44 obtained here is probably the result of reactions (3) and (4) and to a smaller extent reaction (2).

Fig. 1 Phosphate adsorption on hydrous alumina. The curve is the theoretical isotherm obtained by using a two-term Langmuir's equation.



At a $\text{pH} \sim 5$, phosphate in solution is mainly present as monovalent ions and is probably adsorbed on hydrous alumina as monodentate ligands¹. With increasing pH the proportion of divalent phosphate in solution, and consequently that adsorbed, will increase resulting in greater amounts of adsorbed phosphate forming ring structure (4). Phosphate held as six membered ring structure can be expected to be more stable than that bonded as monodentate ligands. Hence the lability of the aluminium phosphate complex would decrease with increasing pH . In fact observation to this effect has been made for the isotopic exchange of adsorbed phosphate on goethite⁸. Although the authors explained their results based on acid-base catalysis, disregarding the phosphate species adsorbed, the present results in conjunction with the earlier report¹ indicate that the charge of the phosphate ion also determines the type of bonding of adsorbed phosphate and therefore its lability.

The change in slope of the hydroxyl ion release data observed at $212 \mu\text{mol}$ of phosphate adsorbed (Fig. 2) suggests a different

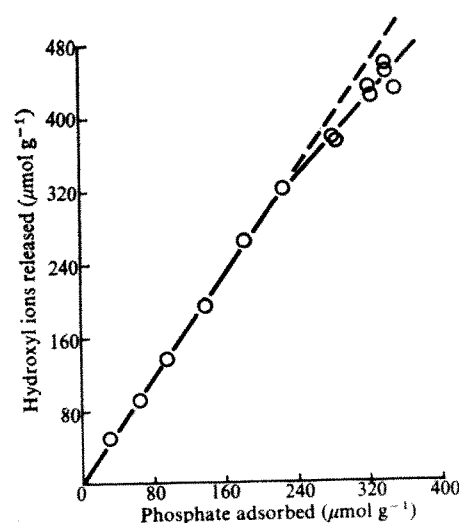


Fig. 2 Hydroxyl ions released plotted against phosphate adsorbed. The two straight lines are drawn down according to the equations:

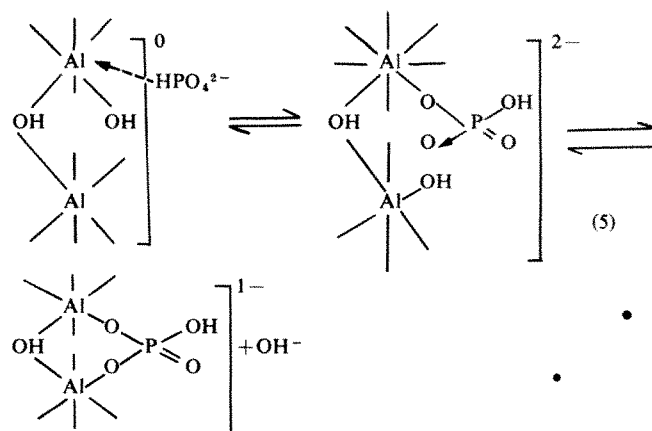
$$\text{OH}^- = 1.44(\pm 0.05)\text{P at } \text{P} < 212(\pm 40) \mu\text{mol}$$

$$\text{OH}^- = 1.07(\pm 0.11)\text{P} + 79.3(\pm 33.4) \text{ at } \text{P} > 212 \mu\text{mol}$$

Confidence interval 95%.

mechanism of adsorption at greater values. The fact that the experimental points at high adsorption values deviate from the Langmuir curve corroborates this suggestion.

The change in slope probably indicates disruption of aluminium polymers¹. As the chemical potential of phosphate in solution increases, the phosphate ions will compete with the OH^- that are bridging aluminium atoms (ol groups) and eventually displace them forming ring structure (5).



The calculated value of R for such a reaction is 1.0 which agrees with the observed value of 1.07.

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Reversibility and biological machines

THE application of the traditional, macroscopic arguments of thermodynamics to very small systems (for instance, using individual cross bridges to generate force in striated muscle, which use "one molecule of ATP at a time"¹), has been questioned². It is demonstrated here that they cannot be applied to very small systems without very drastic modification. That is because of the requirements of quantum theory and information theory. The modifications are substantial and inescapable for systems such as individual cross bridges although they become negligible for macroscopic systems.

Consider a machine capable of performing mechanical work. Parallel considerations apply to a device performing electrical work (for example, a fuel cell) but the distinction becomes blurred at the molecular level. For want of a better term I refer to the point of application of the force as the 'piston', although I may be referring to a part of an individual macromolecule undergoing a conformational change. The quantal definition of work, using the Born-Oppenheimer separation of electronic and nuclear motion, has been treated fully elsewhere (B.F.G. in preparation) in a discussion of the quantum mechanics of cross-bridge action.

If the force function is $F(x)$, where x is the position of the piston, then to perform work reversibly, the position of the 'piston' must be measured accurately in order to apply the appropriate external force F_{ex} . Quantum theory and information theory require that a minimum amount of energy is expended in ascertaining position x to within limits $\pm \Delta x$ (where $\Delta x > 0$). The energy cost ΔE is

$$\Delta E \geq hc/4\Delta x \quad (1)$$

according to Brillouin³, where h is Planck's constant and c is the velocity of light. Obviously, ΔE is completely negligible for a macroscopic observation (that is, if Δx is about 1 mm) but for a molecular system ΔE is very important. The uncertainty in the force generated at position x in the process is then

$$\Delta F = F(x) \pm \Delta x(dF/dx) \quad (2)$$

We can assume without loss of generality that $dF/dx < 0$. To perform a reversible extension, we would normally make F_{ex} as close as possible to F (thus ensuring that $F_{ex} = F(x) - |\epsilon|$, with $|\epsilon| \rightarrow 0$ in the limit). Because of the uncertainty the best that becomes attainable is

$$F_{ex} = F(x) + \Delta x(dF/dx) - |\epsilon| \quad (3)$$

The work done by the system is then, in the limit $|\epsilon| \rightarrow 0$

$$W^+ = \int_{x_1}^{x_2} F(x)dx - \Delta x \int_{x_1}^{x_2} \frac{dF}{dx} dx \quad (4)$$

where $F_1 > F_2$ because $x_2 > x_1$. Conversely, in a compression the best that can be attained for the work done on the system is to take $|\epsilon| \rightarrow 0$ in equation (5)

$$F_{ex} = F(x) - \Delta x(dF/dx) + |\epsilon| \quad (5)$$

so that

$$W^- = W_{rev}^- - \Delta x(F_4 - F_3) \quad (6)$$

where $F_4 > F_3$. Clearly, for any cycle involving j steps

$$W = W_{rev} - \Delta x \sum_j |F_j - F_{j+1}| \quad (7)$$

where the correction is always negative. If the net energy input during the cycle is Q , then the conversion factor θ is given by

$$\begin{aligned} \theta &= W/(Q + \Delta E) \\ &= (W_{rev} - \Delta x \sum_j |F_j - F_{j+1}|)/(Q + \Delta E) \end{aligned} \quad (8)$$

By substituting from inequality (1) this gives

$$\theta \leq (W_{rev} - [hc \sum_j |F_j - F_{j+1}|/4\Delta E])/(Q + \Delta E) \quad (9)$$

which can in turn be rearranged in terms of convenient dimensionless quantities α and z

$$\theta/\theta_{rev} \leq (1 - \alpha/z)/(1 + z) \quad (10)$$

where θ_{rev} stands for W_{rev}/Q , that is, the classical conversion factor; the relative or dimensionless energy cost of control

$$z = \Delta E/Q$$

and

$$\alpha = hc \sum_j |F_j - F_{j+1}|/4QW_{rev}$$

For classical thermodynamics, $\alpha = 0$, that is, Planck's constant is considered negligible, and $z \rightarrow 0$ because the energy cost of achieving reversibility is ignored, whereupon $\theta \rightarrow \theta_{rev}$. For quantal or molecular machines the optimum controlled situation can be calculated by differentiating equation (10) with respect to z (α constant) and setting the derivative equal to zero. The result is

$$z_{max} = \alpha(1 + \sqrt{1 + 1/\alpha}) \quad (11)$$

and this gives the maximum possible value for θ/θ_{rev} as

$$(\theta/\theta_{rev})_{max} = \frac{1 - \{1 + \sqrt{1 + 1/\alpha}\}^{-1}}{1 + \alpha\{1 + \sqrt{1 + 1/\alpha}\}} \quad (12)$$

If more energy than z_{max} is used, then α decreases because of the energy cost of information; if less energy than z_{max} is used θ decreases because of the irreversibility (to dissipation, acceleration of the piston and so on).

For a macroscopic amount of gas, for instance, 1 mol, where $F_j \sim 10^5$ N, and $Q \sim 4 \times 10^4$ J, assuming $\theta_{rev} = 0.9$, $\alpha = 3.5 \times 10^{-28}$ and so, from equation (11), $z_{max} = 1.87 \times 10^{-14}$. (It is important to remember that α and z are dimensionless numbers.) The energy cost of optimal control, Qz_{max} turns out to be

7.5×10^{-10} J, and the resulting conversion factor, θ , is within one part in 10^{14} of the classical value θ_{rev} .

For a cross bridge, or for any comparable molecular machine (because the orders of magnitude are not affected by the detailed mechanics of the system), the situation is very different. Using figures quoted by Huxley⁴ $F_j \sim 10^{-12}$ N, $Q \sim 6.67 \times 10^{-20}$ J (the 'ATP quantum' of energy/molecule), and again taking $\theta_{rev} = 0.9$, then $\alpha = 12.37$ and $z_{max} = 25.2$. The energy cost of optimal control now turns out to be 1.6×10^{-18} J, which is 25 ATP quanta: $(\theta/\theta_{rev})_{max}$ turns out to be 0.018.

Clearly, any degree of control tending towards reversibility is out of the question for systems as small as this, as in fact they are known to operate at much higher conversion factors than 0.018, both on grounds of theory (BFG unpublished) and of experiment⁵.

Accordingly the conclusion must be reached that the reversible operation of a machine is not in general optimal for the conversion into work of energy entering into the system, except in the limiting case of macroscopic systems where the energy cost of the necessary control becomes negligible compared with the output per cycle of the machine. Further, the optimal conversion process must, in general, occur at a finite rate and, for very small systems for which $z_{max} > 1$, this rate is uncontrolled and determined by the mechanics (quantum or semiclassical) of the system itself.

For intermediate cases the optimal rate will be determined by a compromise between the free running system and its control. Only in the classical limit, $\alpha \rightarrow 0$, $z_{max} \rightarrow 0$, is the optimal process infinitesimally slow. It is interesting to estimate that the minimum size of machine discernible and therefore potentially controllable, by the ATP energy quantum is given by inequality (1) as $2\Delta x = hc/2\Delta E \geq 1.4 \mu\text{m}$, which is almost exactly the experimental half-sarcomere length in vertebrate striated muscle, generally regarded as the minimum functional unit under the control of the appropriate Z band.

With regard to the statement of the second law of thermodynamics in a form appropriate to molecular machines, Popper⁶ and McClare⁷ have already stated clearly that the problem involved the necessity of defining work at the microscopic or molecular level. This definition, in purely quantum mechanical terms, is the starting point for Gray and Gonda (BFG in preparation) in a programme of the theoretical investigation of biological molecular machines.

It is necessary to discuss the Born-Oppenheimer separation of nuclear and electronic motion, and the Hellmann-Feynman theorem for time-dependent states. Under certain conditions that are well satisfied in biological machines, it is possible to show that the expectation value of the force as calculated from the Hellmann-Feynman theorem is an adiabatic invariant for non-stationary states involving large nuclear displacements (conformation changes). Work can then be defined simply as a path integral in the usual way. In spite of this, the word 'work' is conspicuously absent from every quantum mechanical textbook I have been able to consult.

From this viewpoint (BFG in preparation) it seems clear that a molecular machine, that is, one which must work below the level of profitable controllability, can operate cyclically and repeatedly in one direction only. If it is regarded as the analogue of a 'heat engine', to use a classical term, it cannot be run backwards as the analogue of a 'heat pump' or 'refrigerator' stretching an actively contracting muscle will not cause cross bridges to traverse their cyclic path in reverse to resynthesise ATP (even assuming the used ADP stayed put) without violating this statement. It is a statement at the molecular level parallel to the macroscopic statement that Joule's experiments on the first law of thermodynamics cannot be carried out in reverse, water will not spontaneously cool and lift a weight.

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Time to understand pictures and words

WHEN an object such as a chair is presented visually, or is represented by a line drawing, a spoken word, or a written word, the initial stages in the process leading to understanding are clearly different in each case. There is disagreement, however, about whether those early stages lead to a common abstract representation in memory, the idea of a chair¹⁻⁴, or to two separate representations, one verbal (common to spoken and written words), and the other image-like⁵. The first view claims that words and images are associated with ideas, but the underlying representation of an idea is abstract. According to the second view, the verbal representation alone is directly associated with abstract information about an object (for example, its superordinate category: furniture). Concrete perceptual information (for example, characteristic shape, colour or size) is associated with the imaginal representation. Translation from one representation to the other takes time, on the second view, which accounts for the observation that naming a line drawing takes longer than naming (reading aloud) a written word^{6,7}. Here we confirm that naming a drawing of an object takes much longer than reading its name, but we show that deciding whether the object is in a given category such as 'furniture' takes slightly less time for a drawing than for a word, a result that seems to be inconsistent with the second view.

In each of three conditions with different adult subjects, 96 line drawings of objects or their names written in lower case Letraset (Berling 14 point) were presented one at a time in a tachistoscope, preceded and followed by a mask of haphazard lines and pieces of letters. Each subject saw half the 96 items as words and half as drawings, in alternating blocks of 16 items. Each item was presented as a drawing to half the subjects and as a word to the other half. The subject had never seen the drawing before it was presented. The experimenter said ready or (in the third condition) named a category before each presentation, and after an 800-ms interval the item appeared. A voice key was used to measure response time from the onset of the item.

To discover whether the drawings and words were equally discriminable as visual patterns, in the first condition 16 subjects were shown the items for brief durations, 40, 50, 60, or 70 ms. The durations were presented in a random order, permuted across subjects so that each item was shown equally often at each duration. Subjects named or read the items. The estimated exposure duration required to report 50% of the items correctly was 44 ms for the drawings and 46 ms for the words.

In the remaining two conditions items were presented for 250 ms, at a level well above threshold. Subjects in the second condition ($n=8$) named the object or the word aloud, as rapidly as possible. In the third condition ($n=16$) the experimenter named a category before the item appeared. The subject said yes if the item was a member of the category, as it was on half the trials, and said no otherwise. Altogether there were 18 categories containing two to nine items: for example, food (carrot, pie), clothing (hat, coat), tools (pliers, hammer).

The results of the second and third conditions are shown in Fig 1. As in earlier reports^{6,7} drawings took longer to

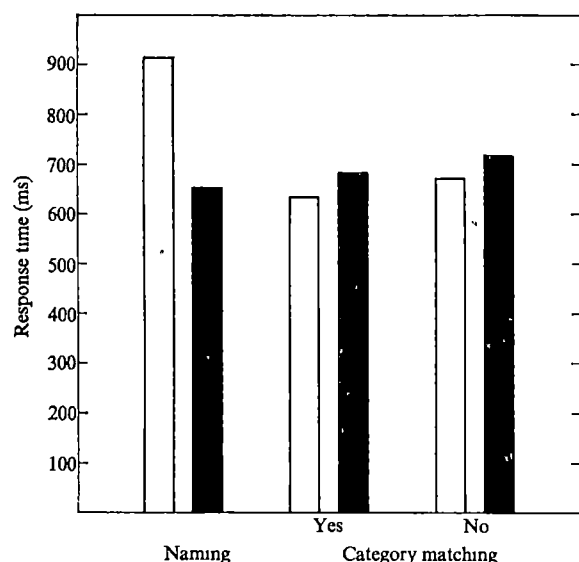


Fig 1 Mean response time in condition 2 (naming) and condition 3 (matching the item to a spoken category). The white bars are responses to drawings, the black bars, to words. Each bar is based on at least 350 responses, errors and responses that took longer than 2 s (together, less than 5% of the trials) were omitted.

name than words, the mean difference was 260 ms (standard error of the mean difference, 91 ms). The difference was in the same direction for all eight subjects ($P < 0.01$, sign test), and for 93 of the 96 items ($P < 0.001$). In the third condition drawings were categorised faster than words: a difference of 51 ms overall (standard error of the mean difference, 42 ms). The difference was 57 ms for yes responses and 44 ms for no responses. Fourteen out of 16 subjects were faster with drawings than words ($P < 0.01$, sign test). Of the 96 items, 68 were matched faster as drawings ($P < 0.001$).

Recall that the second view of memory asserts that an object has two representations, and that an object's category (a verbal abstraction) is associated with its name and only indirectly with its appearance. If, as that view claims, a drawing must be named implicitly before its category is determined, then in the present experiment one would expect drawings to be categorised more slowly than words^{8,9}, just as they were named 260 ms more slowly. But drawings were not slower than words: they were 50 ms faster. Furthermore, a drawing was categorised much more quickly than it was named, which also makes it unlikely that naming preceded categorising. That finding is, however, not by itself conclusive, since a yes-no matching response may be simpler and so faster than overt naming.

Before one concludes that the second view is untenable, the following four objections must be considered.

(1) Drawings in a given category might have shared certain visual features, so a drawing may have been categorised rapidly on the basis of those features before the subject knew exactly what it was⁹. That is unlikely because the items were chosen to look as diverse as possible, and because at near-threshold durations (first condition) subjects rarely reported a drawing's category but not its name.

(2) Some words (for example, bear, tie, train) were ambiguous, and the first meaning assigned by the subject may not have matched the specified category. An analysis of just the unambiguous items, however, reduced but did not eliminate the advantage of drawings.

(3) Concrete words must be imaged to be categorised—the converse of the naming hypothesis. That seems unlikely, since imaging a word is reported to require at least 0.5 s (ref. 5).

(4) The category of an item is independently associated

to both its name and its appearance. Although our results do not contradict that unparsimonious hypothesis, it would be surprising if a verbal category were more strongly associated with a drawing than a name.

The first view claims that written words and drawings (and presumably also spoken words and objects experienced directly) lead to a common representation in memory, neither word-like nor image-like, and it is that representation which is connected with knowledge of an item's category. In our study, that representation was reached more rapidly from drawings than from words. On this view, naming a drawing is slow because it requires an extra step from the abstract concept to its associated name, whereas naming a word only requires that the word pattern itself be identified¹⁰ and then it may be articulated even before the concept is evoked.

In sum, our results are consistent with the view that knowledge of the category of an object is associated with an abstract idea of the object rather than directly with its name or appearance. Since the name and appearance of an object are also represented in memory, a further question is whether other knowledge one has about an object (such as its typical size or value) is linked to the abstract concept or is directly associated with the name or image. The answer may help to resolve an old question: what are the functions of images and words in thought?

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Sex differences in imagery and reading

STANDARD texts on differential psychology often discuss briefly the existence of sex differences in the ability to perform various cognitive tasks^{1–3}. A rough generalisation is that females perform better than males on verbal tasks (for example, verbal fluency, articulation, spelling), whilst males are superior on visuospatial tasks (for example, maze learning or form-board tasks), although exceptions are plentiful. In view of the emphasis in contemporary cognitive psychology on the pervasiveness of verbal coding in a variety of cognitive tasks, and also current interest in such visuospatial abilities as visual imagery or mental rotation, it seems surprising that no attention has been paid to the possible existence of sex differences.

A possible reason for this neglect is that the sex differences observed in psychometric investigations are usually very small, and sometimes unexpectedly absent, as for example in block design tasks, which would seem to be visuospatial^{4,5}. The inability to obtain clear or even consistent sex differences may well be the result of the impurity of the tasks used. Ostensibly verbal tasks such as syllogistic reasoning or counting can be shown to involve visuospatial imagery^{6,7}, and ostensibly visuospatial tasks such as block design could be successfully performed verbally. Consequently a large underlying sex difference in mode of processing would not necessarily reveal

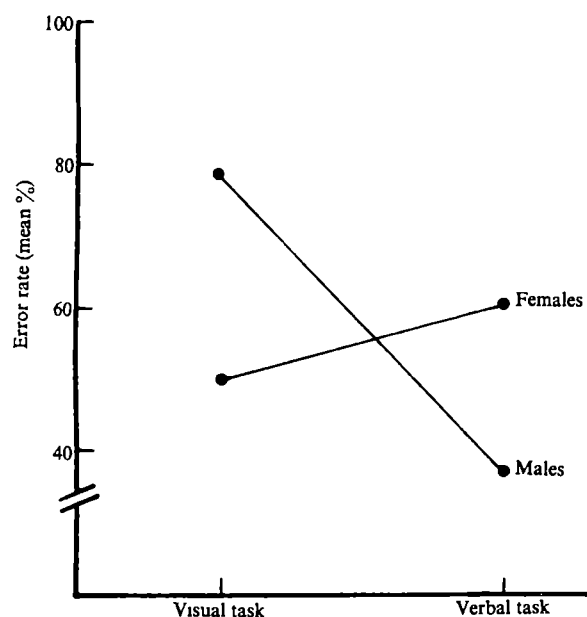


Fig. 1 Percentage of subjects giving the correct response on each task in experiment 1

itself as a sex difference in level of performance in these tasks. A study of the effect of brain damage on performance on the Kohs block design task⁸ illustrates this possibility. In this study, male performance was more impaired by right-hemisphere than by left-hemisphere damage, whereas for females degree of impairment did not depend on site of damage, furthermore, for females with left-hemisphere damage, but for no other group, there was a significant positive correlation between performance on a verbal task (an aphasia battery) and block design performance. This pattern of results is consistent with extensive verbal involvement in block design performance for females but not for males.

What is needed if these issues are to be properly studied are tasks which are purely verbal or purely visuospatial. We used two such tasks in our first experiment. For the verbal task, subjects were asked to proceed mentally through the alphabet from A to Z, counting the number of letters containing the sound 'ee', including E. No external aids such as speaking or writing were permitted. The subjects were asked to perform as rapidly as possible and the time between the beginning of the task and the utterance of the solution was measured. The visual task, performed under the same conditions, was to proceed mentally through the alphabet from A to Z, counting the number of letters containing a curve in their upper-case form. Since no information about the shape of a letter could assist in deciding whether its name contains the sound 'ee', and since no information about the sounds constituting the name of a letter could assist in deciding whether its printed form contains a curve, we considered that these tasks were to a sufficient degree purely verbal and purely visual. The subjects were 75 right-handed undergraduates at the University of Reading, 38 females and 37 males, they were tested individually with both tasks.

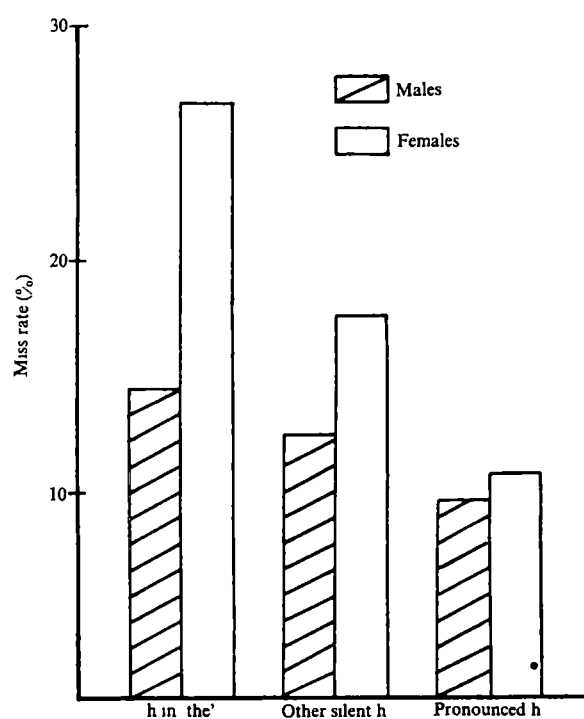
Females completed the verbal task more rapidly than males (22.9 s compared with 23.7 s) whilst males completed the visual task more rapidly than females (21.5 s compared with 22.4 s), but this interaction was not statistically significant, because of the very large within-sex individual differences in completion times. A significant effect was, however, obtained in error rates. Surprisingly, many subjects gave incorrect responses, no doubt because they had been asked to perform the task as quickly as possible and were being timed. The percentage of subjects responding correctly to each task is shown in Fig. 1. Significantly more females than males were correct on the verbal tasks ($\chi^2=3.85$, $P<0.05$). Significantly more males than females

were correct on the visual task ($\chi^2=6.50$, $P<0.05$). The sex difference in error rates is quite large, for both tasks.

To determine whether such marked differences are confined to tasks involving imagery, we next used a task developed by Corcoran⁹. He found that subjects asked to scan through a passage of prose crossing out all occurrences of the letter 'e' overlooked unpronounced e's (as in 'late') much more often than pronounced e's (as in 'let'). This indicates that both verbal and visual analysis is occurring in this apparently visual task. If females rely strongly on verbal analysis, and males on visual analysis, then females should have more difficulty than males in detecting unpronounced letters, since verbal analysis cannot detect such letters. We used 10 male and 10 female undergraduates and asked each to scan through six photographically enlarged pages from novels, crossing out all occurrences of the letter 'h'. They were given 2 min for each page, with 2 min rest between pages. The results are shown in Fig. 2. For pronounced targets, there was a negligible difference between male and female miss rates; for unpronounced targets, female miss rates were much higher than male miss rates. This interaction between target type and sex was significant ($F=4.15$, $P<0.05$) as was the main effect of target type ($F=6.97$, $P<0.01$) and sex ($F=25.50$, $P<0.01$).

This result implies that phonological coding during reading is more prevalent in women than in men, and this was investigated more directly in our third experiment. If we use the term 'internal lexicon' to refer to the stored body of knowledge concerning the words of the language, and the term 'lexical entry' to refer to a subset of the information referring to a particular word (information about how the word is spelled, how it is pronounced, what it means and so on), then the task in visual word recognition is to extract information from a visually presented sequence of letters, and to use this information to locate the lexical entry belonging to the word being viewed (for example by searching the lexicon for an entry containing this information). Given this theoretical framework, we can ask what the nature of the information is. An enduring conflict in the study of reading has been between those who consider that the information is visual and those who consider that it is phonological, that is, that a letter sequence is converted to a phoneme sequence (using a system of grapheme-phoneme

Fig. 2 Miss rates in experiment 2



correspondence rules) before lexical access. There is evidence in favour of both views, this conflicting evidence can be reconciled by proposing that both forms of access are used, simultaneously and independently, and that whichever system locates the required lexical entry first is responsible for word recognition on that particular occasion. Given this, we might expect a sex difference in the relative efficacy of the two systems, word recognition mediated by phonological encoding might be the predominant method of lexical access for women, and visually-mediated word recognition might be the predominant method for men.

We investigated this by using a lexical-decision task¹⁰. On each trial in this experiment, the subject was presented with a row of letters and asked to press a YES key as quickly as possible if the stimulus were an English word, otherwise to press a NO key. The stimuli were presented on an oscilloscope screen controlled by a PDP-12 computer, which also measured the time between display onset and response, recorded which response was made, and printed a data analysis at the end of each subject's session. The subjects were 10 male and 10 female undergraduates and research students at the University of Reading.

A total of 156 letter sequences was presented, in a different random sequence to each subject. Of these, 39 were homophonic words, that is, for each of them there existed another English word with the same pronunciation and a different spelling (for example, SUITE, PAWS or URN), 39 were non-homophonic English words, each matched with one of the homophones on number of letters, number of syllables, word frequency and part of speech, 39 were homophonic non-words constructed so that for each of these non-words there existed two English words with the same pronunciation but different spellings (for example, HORL, LAKS, or THROO), and 39 were non-homophonic non-words, each derived from one of the homophonic non-words by changing one letter so that the resulting letter sequence was still perfectly pronounceable but was not pronounced like any English word.

If access to the internal lexicon is ever phonological, it will be more difficult to say NO to HORL than to DORL, since the phonological system will incorrectly locate a lexical entry in the case of HORL but not in the case of DORL¹⁰. Furthermore, if phonological access is more prevalent in women than in men, then the difficulties engendered by homophonic non-words such as HORL should be more severe for women than for men.

Mean latencies for correct NO responses were 578 ms for men and 737 ms for women when homophonic non-words were used, and 545 ms for men and 647 ms for women when non-homophonic non-words were used.

The NO response was thus slower for non-words which sounded like English than for those which did not. This was true for every one of the twenty subjects. The effect was larger for women than for men (33 ms compared with 90 ms), and this difference was statistically significant (Mann-Whitney $U=23$, $P<0.05$). In the case of words, response latency was not influenced by whether or not a word was a homophone, the only significant effect was that women were about 100 ms slower than men in responding YES to words.

The results with non-words indicate that phonological access to the lexicon is used to some extent by both sexes, but more by women than by men. If phonological access is completed before visual access more often in women than in men (for example, because women are more efficient or faster than men at applying phonological rules to letter sequences), the sound of the word will have more effect on women than on men, as was the case.

Whether these sex differences are present early in life remains to be determined, but if the same pattern of sex differences appears in young children, this finding may have educational implications. For example, it may prove efficient to teach girls to read by a phonological method ('phonics') and boys to read by a visual method (for example, the 'whole-word'

method). Some of the sex differences in cognition discussed earlier may in fact be present very early in life^{2,11}. Furthermore, male superiority in spatial tasks is evident in rats as well as men¹¹, and seems to be susceptible to hormonal influences in rats and in men^{11,12}. If relatively pure tests of visual and phonological processing, such as those used in experiment 1, can be devised which are suitable for use with children before they learn to read, it may be possible to obtain satisfactory evidence concerning the origins of the sex differences in cognition described in this paper.

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A model illustrating the importance of timing in the regulation of breathing

THE effectiveness of afferent volleys from arterial chemoreceptors in stimulating inspiration depends on the precise timing of their arrival within the central nervous system^{1,2,16}. Torrance¹ suggested that variation in the degree of mismatching of the actual with the optimum timing of volleys would provide an extra element of fine control in the respiratory system. I have developed a scheme which is based on this suggestion and also on the following experimental observations.

(1) In the cat the afferent impulses from chemoreceptors are grouped in bursts the peaks of which correspond in time to the middle of the rising phase of the oscillations of carotid arterial P_{CO_2} (refs 3 and 4), peak afferent discharge should thus be related to mid-expiration, the two events being separated in time by the lung-to-carotid body circulation time, itself relatively constant under a single set of circumstances, and in normal men commonly occupying the duration of nearly two respiratory cycles⁵.

(2) A burst of chemoreceptor discharge in the cat is only effective in augmenting inspiration if it falls within a certain part of the cycle, the period of central inspiratory excitability (PCIE), which is closely related to inspiration itself. Inspiration cannot be excited by this pathway during the rest of the cycle, the system behaving as if memory is short^{1,2}. Afferent volleys arriving outside the PCIE do, however, prolong expiration^{1,16}.

(3) In man, the respiratory system is capable of detecting differences in the alveolar P_{CO_2} time profile so brief as to occupy only one-third of the whole cycle. This property is almost certainly mediated by arterial chemoreceptors⁶.

(4) Also in man, with changes in chemical drive from any source, tidal volume (V_T) and the duration of expiration (T_E), and hence total cycle duration (T_T) are inversely related⁷. The inverse relation holds good on a breath-by-breath basis⁸.

A crude model has been constructed (Fig 1) using squared paper and following some arbitrary quantitative rules: (a) initially steady eupnoeic inspiration and expiration occupy 1 and 2 s respectively, (b) PCIE coincides exactly

with inspiration, (c) the afferent volleys also last for 1 s, (d) a volley stimulates inspiratory volume and shortens expiratory duration in proportion to the overlap between it and PCIE, and (e) with no overlap the volley is ineffective, inspiratory volume is decreased and expiratory duration lengthened from the eupnoeic values. In these circumstances, the residual ventilation is prevented from falling below 70% of the eupnoeic level by the slowly-acting central drive from (CO_2 , H^+)

In cases 1 and 2 (Fig 1) the lung-to-carotid body circulation time is such that, with the initial steady breathing, the burst of afferent impulses follows the PCIE of the next-but-one respiratory cycle and overlaps it by one-fifth. In cases 3 and 4 the circulation time is lengthened so that the afferent burst is delayed sufficiently to overlap the PCIE of the next-but-two eupnoeic cycle, the overlap, however, although again one-fifth, is reversed and the burst precedes the PCIE.

After four steady eupnoeic cycles (Fig 1) an extrinsic irregularity, comprising a long (cases 1 and 3) or short (cases 2 and 4) breath, disturbs the rhythm, such irregularities occur continually in normal human breathing⁹⁻¹¹. The regular time-base provided by the spacing of the afferent bursts is not disturbed until two cycles later, but the overlap between the bursts and the PCIE is altered after only one cycle, the consequences for \dot{V}_T and T_E depend on the circumstances (Fig 1).

In cases 1 and 2, after the disturbance, a large breath of short duration alternates with one or two small breaths of long duration but the alternation of the overlap between afferent bursts and PCIE is such that correction by the timing mechanism is towards the mean, and regularity (case 2), or regular non-uniformity (case 1), of the time base is quickly re-established. Over two or three cycles in cases 1 and 2, respectively, the mean quotient $\dot{V}_T/(T_I + T_E)$, (=mean ventilation, \bar{V}) remains at the predisturbance value. In other words, negative feedback results and overall stability of ventilation is achieved.

On the other hand in cases 3 and 4 (afferent bursts preceding PCIE), the initial steady eupnoea turns out to be unstable. After an extrinsic long breath (case 3), PCIE falls so far behind the afferent burst as to lose touch completely and the mean ventilation stabilises at the minimum value of 70% of eupnoea, the time-base is slowed and is incapable of reversing the situation. After an extrinsic short breath

(case 4), the burst-PCIE overlap is increased and the next breath is also shortened. A complex oscillation of response ensues, with the pattern repeating every six breaths, the time-base is irregular, mean ventilation settles 33% above the eupnoeic value and the time relation between burst and PCIE may reverse. Although the details of the pattern depend on the numerical values assumed, the general features are found with most of the likely combinations. Cases 3 and 4 exhibit an element of positive feedback, limits being set by the maximum and minimum possible effectiveness of the chemoreceptor drive under the prevailing conditions of blood-gas tensions.

Models of this type are consistent with a number of observable phenomena of normal human breathing. The enhancement of hypoxia sensitivity in even mild exercise¹² in the absence of any increase in the average impulse frequency in the chemoreceptor afferents¹³, could be due to 'sharpening' of the grouping of impulses into bursts. Stability of ventilation would, however, require that the PCIE precede the bursts, as in cases 1 and 2.

At low ventilations, T_E varies much more with small changes in \dot{V}_T than when ventilation is high⁷. The model would thus predict the well-known irregularity of breathing in the short term during eupnoea, especially when inhalation hypoxia is present. It also predicts the greater regularity when ventilation is stimulated by CO_2 inhalation, although other factors are certainly involved here.

In the periodic breathing of high altitude the period of the swing is about 0.75–1 min, a time more appropriate to the slow, well-damped negative-feedback loop involving the intracranial (CO_2 , H^+) receptor system, than to the fast, poorly-damped loop involving peripheral chemoreceptors. The latter, however, is an essential component as its inactivation by breathing oxygen abolishes the periodic breathing. A scheme like that of cases 3 and 4 would resolve the paradox with the afferent bursts preceding PCIE, a slight disturbance would promote the maximum or minimum situation, with ventilation and metabolism mismatched. Correction of the mismatching by the central (CO_2 , H^+) loop would have a long time constant but the correction itself would activate the peripheral loop which, by positive feedback, would quickly flip the whole system over into the other extreme state. Periodic breathing is most marked in recumbency, especially in sleep, during which lung-carotid circulation time is slightly lengthened and there are few

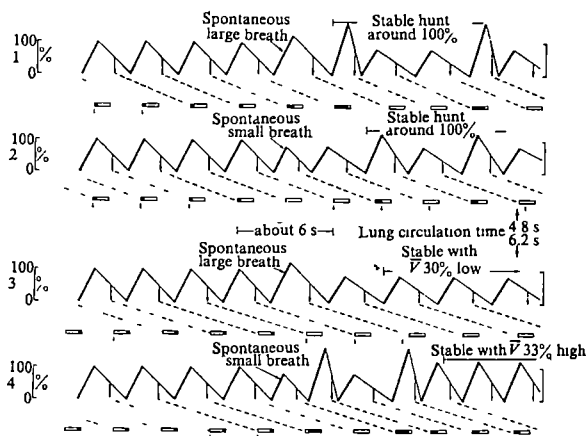


Fig. 1 The timing model simulated time relation between respiratory movements, scaled to percentage of control, and chemoreceptor bursts of activity. Inspiration upwards (bold lines) with four steady eupnoeic cycles at left of each line. Afferent volleys (rectangles) last 1 s and each is connected by dashed line to the middle of the expiration (downward arrow) that initiated it. Volleys only stimulate inspiratory volume and flow, and shorten expiratory duration when they overlap PCIE, which coincides with duration of inspiration. The degree of overlap is indicated by blackening of the volley symbols: + $\Delta 0.2$ s of overlap results in a + $\Delta 20\%$ tidal volume and - $\Delta 20\%$ expiratory duration. Inspiratory duration (1 s) is unaffected. With no overlap, chemoreceptor bursts are ineffective and central respiratory drive prevents ventilation from falling below 70% of eupnoea. Cases 1 and 2: circulation time is 4.8 s, that is, slightly less than the duration of two respiratory cycles, thus volley overlaps PCIE from behind. An extrinsic long or short breath initiates changes in overlap which do not change mean ventilation, \bar{V} , although in the absence of damping in the model the short term hunt would continue indefinitely. Cases 3 and 4: circulation time is 6.2 s, that is, slightly longer than the time for two respiratory cycles, thus volley overlaps PCIE from in front. An extrinsic long or short breath is followed by changes that reveal the intrinsic instability. In case 3, the time-base provided by the volleys is lengthened and mean ventilation falls. In case 4, the volley-PCIE overlaps vary widely, breathing is irregular and mean ventilation high. These states continue until corrected by slow changes in the central drive, over-correction and longer term hunting are likely to occur.

irregularities to disturb the hunting pattern, although other factors are probably involved. It is also possible that some pathological states result from disorders of the mechanism described.

The model is clearly over simplified: thus PCIE and the afferent bursts have been given duration but not intensity. The time profile of the PCIE might resemble that of the phrenic neurogram and the profile of the bursts is probably like a skewed sine wave⁴. The bursts could also vary in amplitude, and rhythmic or periodic cardio-respiratory events could affect the transit times of the blood-gas oscillations from lungs to receptors. Further developments will require more sophisticated computation.

An important feature of the mechanisms whereby phasic afferent activity could produce the effects described is that there should be little or no central memory of earlier bursts, this could be achieved if the connections were very simple. Indeed, Davies and Edwards¹⁴ found few synapses between the incoming fibres and the central inspiratory neurones. The nature of the effect on T_E is not known. Clark and Euler¹⁵ report that induced changes in V_T lead to changes in T_I , which they regard as being linked to T_E . In man, however, T_E may respond to changes in chemical drive while T_I remains constant⁷.

The relative contribution of the mechanism to short term regulation in normal man at rest at sea level is probably small and possibly even trivial^{5,12}. During exercise, however, 'sharpened' bursts of afferent activity could provide a back-up device such that if ventilation, and particularly its frequency component, were to fall momentarily below the requirements of metabolic exchange, both would receive an extra 'push from behind' so that the proper separation between afferent burst and PCIE would rapidly be re-established.

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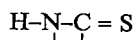
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PTC taste blindness and the taste of caffeine

TASTE thresholds for the bitter substance PTC (phenylthiourea or phenylthiocarbamide) and related compounds containing the grouping



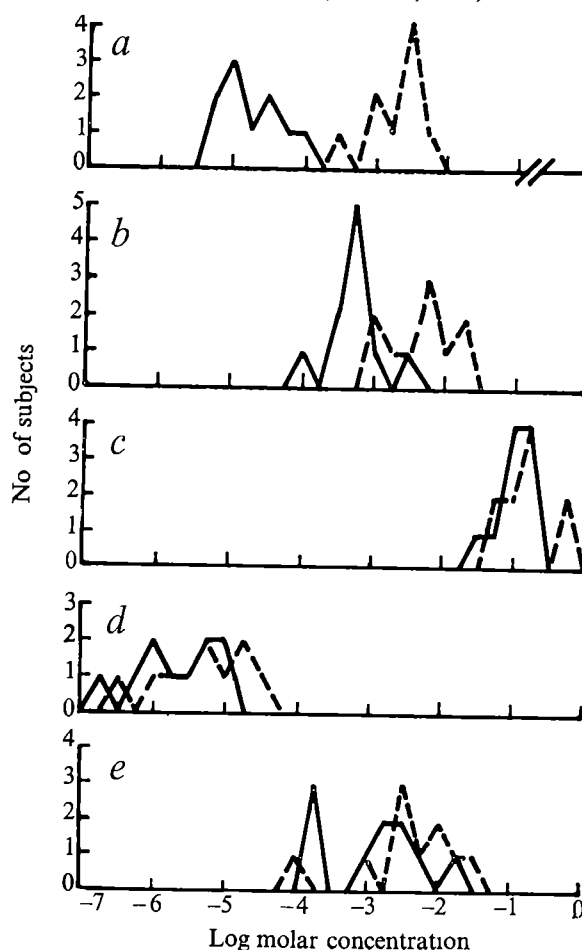
(HNCS) show a bimodal distribution, leading to the designation 'tasters' for the more sensitive individuals and 'non-tasters' or 'taste blind' for the less sensitive. Genetic and population studies have generally attributed this to a simple Mendelian dominance system in humans, non-human primates and some rodent species. All other bitter and non-bitter compounds tested so far have produced Gaussian distributions of thresholds^{1,2}. We have found now, however, that sensitivity to the taste of PTC predicts sensitivity to caffeine, a common bitter substance that lacks the HNCS grouping. This was shown by

threshold measurements and magnitude estimation of supra-threshold concentrations.

We tested ten non-tasters (the first ten undergraduates uncovered by a rough PTC threshold screening procedure) and ten tasters. Each subject's threshold was measured for each of five compounds: PTC, quinine hydrochloride (QHCl), urea, caffeine and sodium chloride (NaCl). The method was a modified Harris-Kalmus procedure³ in which the subject was given eight cups, four contained water (Hydro Service ultrapure water with resistance greater than 18 MΩ cm⁻³) and four contained a particular concentration of the substance under evaluation. The subject rinsed his mouth thoroughly with water before sampling from each of the eight cups which he sorted into two groups: those with a taste and those without. The concentration was decreased successively by 0.25 log steps until the subject failed to sort correctly.

Suprathreshold concentrations were flowed on to the extended tongue at a rate of 4 ml s⁻¹ through a McBurney gravity flow system⁴. The stimuli were always preceded by a 20 s water rinse and were presented, twice each, in random order. Subjects were asked to assign numbers proportional to the perceived intensities of the stimuli⁵. For example, if the first stimulus was judged '10 bitter' and the second stimulus seemed twice as intense, then it was to be judged '20 bitter'. Solutions in both phases of the experiment were warmed to 34°C. Since NaCl and PTC sensitivity are not related, judgments of the bitter substances were expressed relative to NaCl in the following way. The geometric mean of the magnitude estimates for the four NaCl concentrations was obtained for each subject. This mean for each subject was made equal to ten by multiplying with an appropriate factor. The subject's magnitude estimates of the bitter substances were then multiplied

Fig 1. Harris-Kalmus thresholds for PTC (a), caffeine (b), urea (c), QHCl (d), and NaCl (e) for tasters and non-tasters of PTC. *One subject was unable to taste even 0.018 M PTC, the highest concentration used. —, Tasters; ---, non-tasters.



by the same factor. This transformation had no effect on the ratios among magnitude estimates. It merely decreased variability contributed by the absolute size of numbers chosen by the subjects.

Figure 1 shows the thresholds for tasters and non-tasters of PTC. The bimodal distribution for PTC with peaks at 0.00001 M and 0.003 M PTC is typical. The bimodal distribution that resulted for caffeine, however, has not been reported before. Even more interesting, the caffeine thresholds were correlated with the PTC thresholds (Spearman rank correlation coefficient = 0.83, $P < 0.001$). None of the other substances produced a clear bimodal threshold distribution nor were the thresholds correlated with those for PTC.

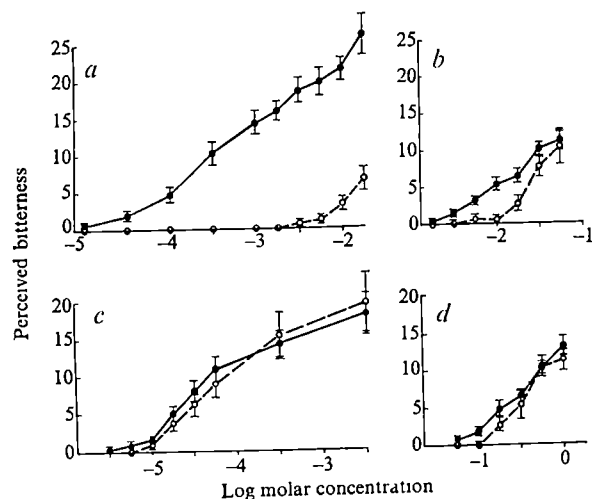


Fig 2 Perceived bitterness of PTC (a), caffeine (b), QHCl (c), and urea (d) for tasters and non-tasters of PTC. ●, Tasters; ○, non-tasters

Figure 2 shows the perceived bitterness of suprathreshold concentrations of PTC, caffeine, urea and QHCl for tasters and non-tasters of PTC. The functions for PTC show the large difference in sensitivity between tasters and non-tasters to be expected from the PTC threshold results. The functions for caffeine also confirm the lesser difference between tasters and non-tasters shown by the caffeine threshold results. The difference is, however, restricted to the lower concentrations (t tests, $P < 0.01$ for 0.056 to 0.03 M caffeine). The steepened slope of the non-taster caffeine function is reminiscent of the recruitment of loudness in typical nerve deafness⁶. At sufficiently high concentrations, the two PTC functions might meet as the caffeine functions do, however, this possibility cannot be tested because of the limited solubility of PTC.

The lower concentrations of urea were also perceived as slightly less bitter by the non-tasters (t tests, $P < 0.01$ for 0.1 and 0.18 M urea). This suggests that the taste of urea, like that of caffeine, may be related to the taste of PTC even though the peaks of the urea threshold distributions for tasters and non-tasters cannot be resolved. Neither caffeine nor urea contains the HNCS grouping. There are many bitter substances that have not been examined for possible connection with PTC sensitivity, so it now seems likely that there must be others that will also taste less intense to PTC non-tasters.

Beidler and Gross⁷ have argued that current knowledge about the nature of taste receptor sites leads to the conclusion that "there exists no single receptor site peculiar for all molecules representative of any particular taste quality". Our psychophysical results add evidence for the existence of at least two receptor sites underlying the taste of bitter, with the 'PTC site' largely absent in non-tasters and the 'quinine site' present in

both tasters and non-tasters of PTC. Caffeine and possibly urea seem to stimulate PTC sites. This interpretation is also supported by cross-adaptation results reported by McBurney and colleagues. Adaptation to QHCl does not substantially reduce the bitterness of PTC (ref. 8) although among sweet, sour and salty substances, cross-adaptation is virtually complete^{9,10}. That is, adaptation to a substance of one of those qualities reduces the taste intensity of other substances of the same quality.

The possibility of multiple receptor sites underlying a single taste quality poses an interesting problem in sensory coding. Why should PTC and QHCl both taste bitter if they are coded by different receptor sites? The cross-fibre patterning hypothesis^{11,12} gives one answer. Both substances may stimulate largely the same population of nerve fibres even if the receptor sites by which they accomplish this stimulation are different. That is, quality is determined by the pattern of neural activity received by the central nervous system.

A series of earlier studies seemed to support the conclusion that a taster needed his own saliva to taste PTC¹³⁻¹⁶. In the classic study, Cohen and Ogden¹⁵ dried the tongues of tasters with air from an atomiser. Although this procedure rendered their tasters insensitive to PTC, it also rendered half of their control subjects insensitive to saturated solutions of saccharin and salt. They concluded that "saliva aids in many taste sensations" but they could have concluded instead that air drying the tongue interferes with normal taste perception. In all phases of our experiment, PTC was tasted after a thorough water rinse. Our threshold values are similar to those obtained without a rinse condition^{1,16}. We conclude that saliva is not crucial to the ability to taste PTC.

One implication of our results concerns the bitter taste of caffeine in coffee. Coffee tastes bitter because it contains several bitter constituents. The concentration of the caffeine in an average cup of brewed coffee, 0.003-0.004 M caffeine¹⁷, is below or near the threshold of most non-tasters but above the threshold of most tasters. Caffeine cannot, therefore, add a significant amount of bitterness to the already bitter taste of coffee for the non-taster of PTC. It may or may not add some bitterness for tasters depending on the intensities of the other constituents. Fisher and Griffin¹⁶ have shown that taste sensitivity to some bitter compounds is correlated with systemic pharmacological sensitivity. It would be particularly interesting to see if taste sensitivity to caffeine predicts sensitivity to the pharmacological effects of caffeine as a stimulant.

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Processing of positional information in the human visual system

CONTOURS seem to be shifted away from the edge of a previously seen figure. Ganz¹ considered this apparent displacement to be caused by lateral inhibition between the contours of an after image of the inducing figure and the test figure itself

Similarly, two lines of different orientation that are presented simultaneously seem to be displaced from one another in orientation. To account for the perceptual expansion of acute angles Blakemore, Carpenter and Georgeson² postulated a superposition of excitatory and inhibitory distributions of activity among the population of orientation detectors in the visual cortex. Superposition would cause the peaks of the distributions to be shifted slightly apart and hence cause the brain to deduce that the angle is larger than it is

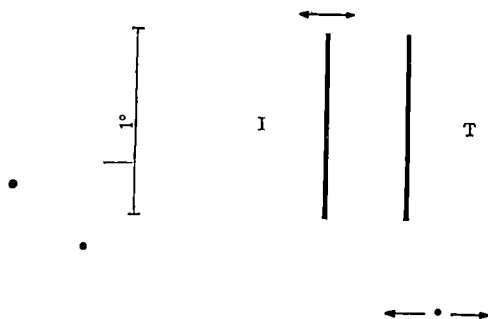
Two parallel lines at threshold facilitate each other when they are separated by less than say 2.5', whereas inhibition occurs for larger separations up to about 10' (ref 3). A line weighting function may be computed from the change of increment threshold for one line as a function of separation from the additional line. The shape of this function compares fairly well with other estimates of the spatial extent of excitatory and inhibitory interactions in the light-adapted fovea.

Line weighting functions may be tentatively ascribed to the combined spread of excitation and inhibition among line detectors. Kulikowski and King-Smith⁴ presented evidence that there exist line detectors which are distinguishable from grating detectors, the 'grating sensitivities' of the two detectors as revealed by subthreshold summation are different. This result receives support from the observation that frequency selective preadaptation of line detecting mechanisms shows no interocular transfer as it is found following adaptation of grating detecting mechanisms⁵. Repulsion between parallel lines separated by less than 10' would then be expected by analogy with the model of Blakemore *et al*. We performed an experiment to test this prediction

The stimuli were two parallel, vertical lines, which were 1° long and 1' wide at a viewing distance of 114 cm, presented on a 6° square background, which had a luminance of 36 cd m⁻² (Fig. 1). The position of the inducing line (I) was set by the experimenter, after which the observer adjusted the horizontal position of a pair of 1' diameter lights, which were 0.5° above and below the test line (T), until they appeared to be aligned with T

The observer, whose position was stabilised by a chin rest, was allowed to inspect freely the test field during his observations. Starting alternately from different directions, it usually took the subject seconds to set the dots. He gave two readings

Fig. 1 Stimulus configuration: the separation between the test line T and the inducing line I was set by the experimenter. The subject had to assess the position of T by aligning the dots and the line



for each position of a series of increasing or decreasing line separations. The procedure was repeated in reverse order. Thus four values were obtained for each position of I in one experimental session

Contrary to expectation, no displacement effects were obtained when test and inducing lines were of equal luminance (Fig. 2a and b). But, for line separations of less than 10' of arc, when the luminance of I was higher T seemed to be displaced towards I (Fig. 2c). An exploratory experiment revealed that this attraction increases monotonically as the luminance difference between inducing and test line increases. For larger separations of the lines one of the subjects (IR) produced two data points with significant ($P \leq 0.05$) values of repulsive displacement whereas the data of another subject (WG) do not show any effect. Standard errors are of the order of magnitude that is to be expected when using an alignment criterion

The basic findings of our experiment have been confirmed

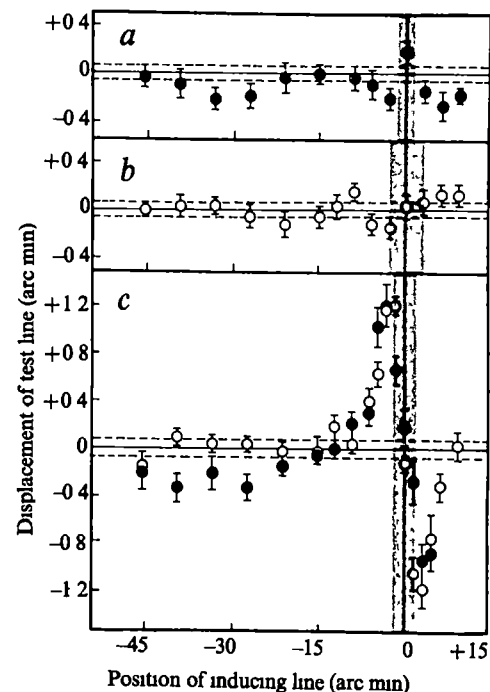


Fig. 2 Changes in apparent position of test line T against distance between T and inducing line I. a, Both lines 0.6 log units above threshold, b, both lines 1.6 log units above threshold, c, test line 0.6 log units, inducing line 1.6 log units above threshold. ●, subject, IR, ○, subject WG. Negative values of displacement mean repulsion at negative values of position (I left of T) and attraction at positive values of position (I right of T). Apparent position of T in absence of I is taken as reference value. Vertical bars \pm s.e. ($n = 12$). Dashed lines indicate \pm s.e. of reference value ($n = 24$). Vertical shaded areas denote where the lines could not be resolved

for two other subjects. To investigate possible influences of the configuration of the comparison stimulus we also performed measurements using a vernier alignment criterion. The vernier line had a length of 1° and was positioned 0.5° below T. The results of this experiment were indistinguishable from those of the main experiment.

Purely attractive displacements such as those observed in our experiment are readily explained by the model of Blakemore and colleagues, if it is assumed that only excitatory components interact for the assessment of position. But an appreciable shift occurs only if the individual distributions differ considerably in height. The lower peak is then displaced toward the larger one, provided that the separation of the lines is not too large. To account qualitatively for the observed range of interactions (about 10'), it must be assumed that the spread of excitatory activity is wider than the centre of sensitivity functions that are derived from threshold measurement³.

Though there are no inhibitory interactions in the assessment

of position, two lines separated by several minutes of arc clearly inhibit each other. A control experiment using the same apparatus showed that both subthreshold and superthreshold inducing lines raise the threshold for the detection of a test line located 6' away. This suggests that lateral inhibition participates in the neural analysis of contrast (for example, threshold detection) but not in that of position. Thus we are led to the conclusion that the detection of line stimuli and the neural evaluation of their position are performed separately in the human visual system. This receives support from the finding⁵ that the accuracy for positional judgments is an order of magnitude less than the accuracy for shape or orientation discrimination, for which virtually all the information available at the retina is used.

Hubel and Wiesel^{6,7} advanced the idea of an hierarchical organisation of the visual system suggesting a convergence of cortical simple cells on complex cells whose positional selectivity is poor. Psychophysical line detecting mechanisms may be correlated with the activity of monocularly driven simple cells³. If the hierarchical model is correct, the tapping of positional information before the convergence of the output of these cells on complex cells would be necessary to prevent the loss of that information.

Our data are not easy to reconcile with the general conclusion of Ganz¹ that parallel contours repel each other when they are brought close together. By this principle Ganz explained an important class of geometrical illusions (for example, Poggendorff, Hering, Zoellner). Our results do not support this hypothesis as we found no displacement at all for parallel lines of equal brightness.

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Retinotectal mismatch: a serendipitous experimental result

THERE is increasing evidence to indicate that neurones are capable of making connections with sites other than those with which they normally connect¹⁻⁵. During an experiment to test the effect on the retinotectal projection of upsetting the normal sequence of innervation of the growing optic tectum by a growing eye in *Xenopus*⁶, we observed, unexpectedly, a result in one animal which could shed some light on a current controversy concerning the manner in which the formation of selective synaptic connections may be controlled. Because this result was fortuitous and because it is difficult to replicate the exact experimental situation we report the result here.

The right eye had been removed at stage 29 (ref 7), before the eye had differentiated, and the animal grew up with only one eye, the left, which innervated the right optic tectum. Two months after metamorphosis the left optic nerve was exposed and cut in the region where the optic chiasma would have been in a normal animal. The optic fibres were then deflected to feed directly into the left optic tectum by placing a small piece of Millipore filter in a midline position. The filter was successful in forcing fibres from the whole eye to innervate the ipsilateral (left) tectum, but it did not

prevent some optic fibres from regrowing to the contralateral (right) tectum. In most cases fibres from the whole eye grew directly to both tecta, as shown by electrophysiological mapping of the retinotectal projection 6 months after the deflection of the optic nerve.

In one animal, however, a most intriguing result was obtained (Fig 1). The projection from the left eye to the left tectum showed the whole nasotemporal extent of the superior visual field covering the dorsal tectal surface—the map was essentially a normal contralateral map, now occurring on the ipsilateral tectum because of the deflection of the optic fibres into this tectum. The same left eye also projected directly to the right optic tectum but in this case the field projection involved only the temporal half of the superior visual field, yet this half-field projected across the whole extent of the dorsal surface of the right tectum. Fibres from only the nasal half of the retina of the left eye seem to have surmounted the millipore barrier and established contact with the right tectum. These nasal retinal fibres (equivalent to the temporal field) in the normal animal occupy only the caudal portion of the tectum and in this animal they occupied only the caudal area of the left tectum. Yet, fibres from this same nasal retina spread their connections across the entire rostrocaudal extent of the right tectum.

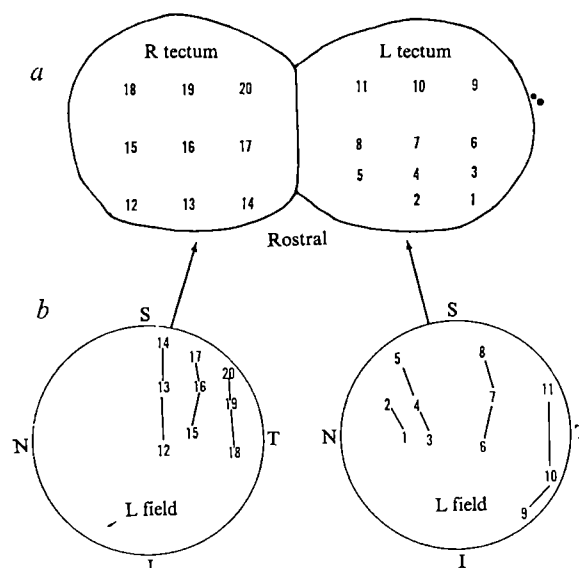


Fig. 1 The projection of the left visual field on to the right and left optic tectum. *a*, The two tecta seen from above, *b*, perimetric charts of the left visual field. The animal should be considered as sitting behind the chart, which covers the visual field from the fixation point out to 100° radially. The numbers on the tectal diagrams represent electrode positions and the numbers on the perimetric chart indicate corresponding field positions. S, Superior, I, inferior, N, nasal, T, temporal.

There have been two different explanations offered to account for previous experimental situations in which half of the retina had been observed to spread its projection over the entire tectal surface⁸⁻¹². One suggestion was that following the operation the nature of the remaining half retina changes and the cell labels which are believed to be responsible for selective neuronal connections^{13,14} are altered. The range of such labels normally associated with a whole retina may thus come to reside in the remaining half retina and this could be the reason that fibres from such a partial retina spread over a whole tectum. An alternative view^{12,15} of the manner in which neurones interconnect argues that the molecular interactions between neurones are essentially competitive, such that the presynaptic fibres compete for the available postsynaptic space.

* On this view the fibres from a half retina might be expected to innervate a complete tectum even though the cell labels in the half retina had not changed

We have here a situation where a half retina, which, because it is half of a known normal eye, should have undergone no reorganisation of its cellular labels, innervates a tectum and covers its whole rostrocaudal extent. This finding constitutes support for the competitive variant of the neuronal specificity theory, in which cellular labels do not, of themselves, determine the tectal site at which a retinal fibre will synapse, but rather fix the relative position within the pre- and postsynaptic arrays that the fibre will adopt. We conclude, therefore, that half a retina which undergoes no reorganisation of its neuronal specificity labels may yet spread its connections in order over an entire tectal surface. Our suggestion that specified neuronal arrays produce selective neuronal connections by a process of competition seems to be supported.

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Re-establishment of functional connections by regenerating central adrenergic and cholinergic axons

AFTER lesion of their axons, central monoamine-containing neurones have a high capacity for regenerative sprouting^{1–4}. This is observed after a mechanical or electrolytic lesion in the brain or the spinal cord as a development of numerous newly-formed axon sprouts extending from the proximal ends of the lesioned axons. These regenerating sprouts can grow to considerable distances, for example, along myelinated fibre tracts and intracerebral vessels^{1–3}. After selective destruction of the serotonin-containing neurones in the central nervous system (CNS), induced by the neurotoxic drug 5,6-dihydroxytryptamine, the serotonin axons regenerate efficiently and in these favourable conditions it has been possible to demonstrate that lesioned central axons can grow back to their original sites of termination⁴.

With the fluorescence histochemical technique the remarkable regenerative capacity of adult central monoamine neurones is also revealed when lesioned noradrenergic neurones are allowed to 'reinnervate' a transplant of peripheral tissue placed in the brain or spinal cord^{2,3}. In this experimental situation the regenerating central noradrenergic sprouts will reconstitute a terminal pattern in the surviving parts of the grafted tissue that closely mimics its original peripheral noradrenergic innervation. Interestingly, this property does not seem to be unique for the noradrenergic neurones in the brain, in that also presumed central cholin-

ergic neurones have recently been found to have very similar regenerative characteristics (our unpublished observations).

Although the regenerated terminal networks are long lasting, microscopical techniques have not yielded any information as to whether the regenerated central axons reform functional synaptic contacts. We have investigated this question in transplants of the portal vein 'reinnervated' by central noradrenergic and cholinergic neurones in the rat brain. We now report that the regenerated central axons form functional connections with the smooth muscle cells in the transplant. To our knowledge this is the first time a re-establishment of functional connections has been demonstrated after axonal regeneration in the adult mammalian brain.

The experiments were carried out on adult female Sprague-Dawley rats (180–200 g body weight at the time of transplantation). Homologous transplantations of the portal vein to the caudal diencephalon were performed by free hand as described previously for transplantations of other tissues^{3,5}. All recipient animals were subjected to cranial sympathectomy through bilateral extirpation of the superior cervical ganglia to exclude any possible interference by peripheral sympathetic fibres in the transplant. Sections of the portal vein, about 7–8 mm long, were opened by a longitudinal cut and the whole or half of the circumference was used. The portal strip thus obtained was placed vertically in the caudal diencephalon of the recipient rat, extending dorsally through the hippocampus and the corpus callosum³. Transplants of the iris which were placed in this position have previously been found to be invaded by regenerating central noradrenaline (NA)-containing and acetylcholinesterase (AChE)-positive fibres (ref. 3 and our unpublished work).

At 25–30 d after operation, the animals were killed by decapitation under light ether anaesthesia. The transplant was removed from the brain and mounted for combined isometric recordings and electrical field stimulation in a 50 ml organ bath as described previously^{6,9}. The buffer was a modified, oxygenated Krebs solution⁹ kept at 38°C throughout the experiment. After an accommodation period of 15–30 min, during which the spontaneous myogenic activity of the transplant was registered, the contractile responses to electrical field stimulation and to exogenous application of neurotransmitters (NA and acetylcholine (ACh)) were studied. Field stimulation was done with square wave impulses of 15 V amplitude between the electrodes (5 mm apart), 10 ms duration, and 6 or 10 Hz. The stimulation was applied for 1 min periods at intervals not less than 5 min. NA or ACh was added to the bath to a concentration of 10⁻⁶ M, and was washed out after 1–2 min. Spontaneous activity was allowed to recover between the responses to drugs or field stimulation. The effects of NA and field stimulation were tested before and after applications of the α -adrenergic blocker phenoxybenzamine (PBZ) (Dibenzylamine, Smith, Kline and French, 10⁻⁶ M) and this was followed by a similar second sequence of experiments comprising exposure to ACh and field stimulation before and after the application of atropine sulphate (10⁻⁶ M). On some of the investigated transplants the experiment was done in the reverse order, that is, starting with the cholinergic receptor blockade, followed by the adrenergic blockade.

The regeneration of NA-containing and AChE-positive nerves into the portal transplants was investigated microscopically with the Falck-Hillarp fluorescence method¹⁰ and then with the Holmstedt¹¹ modification of the Koelle method for AChE, respectively. In the AChE-staining, incubation time was 4–6 h and Mipafox (4 × 10⁻⁶ M) was used as inhibitor of nonspecific cholinesterases. The histochemistry was carried out on serial sagittal sections of portal vein transplants *in situ*. The isolated transplants used in the field stimulation experiments were subsequently pro-

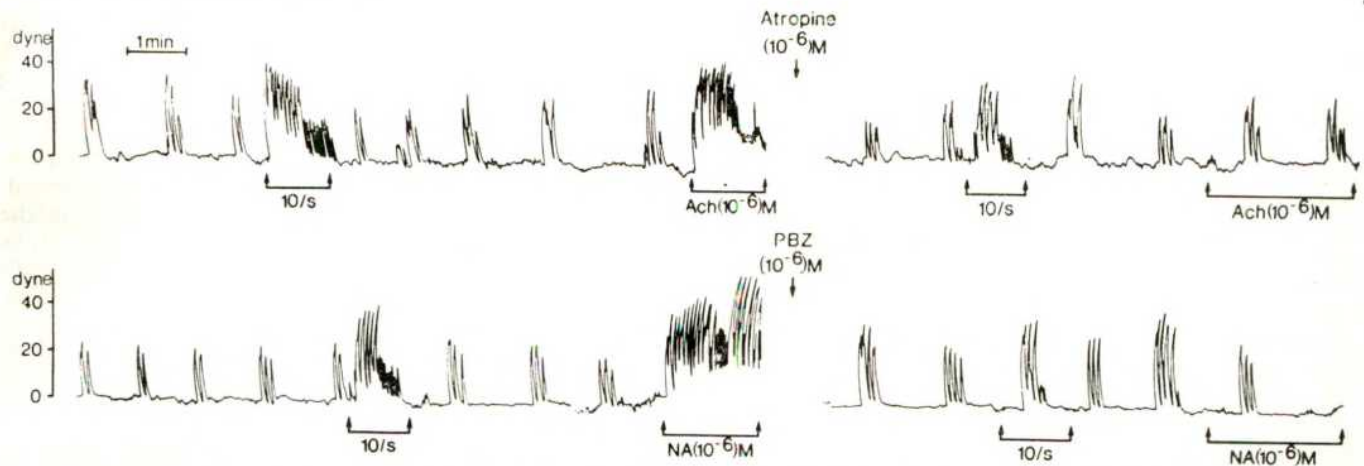


Fig. 1 Spontaneous activity and responses to field stimulation and drug application in a strip of portal vein transplanted to the region of the hippocampus and the caudal diencephalon, 30 d survival. Atropine reduces the response to field stimulation and eliminates completely the effect of ACh. The remaining, atropine-resistant component of the electrical response and the excitatory action of NA are abolished by PBZ. We conclude that in this specimen both cholinergic and adrenergic central neurones have established functional connections with the smooth muscle.

cessed for fluorescence histochemistry of adrenergic nerves.

In its position in the caudal diencephalon and the hippocampus, the portal vein transplant was invaded by regenerating fibres from the locus coeruleus NA axons, running in the so-called dorsal tegmental bundle^{3,6,7} and by AChE-positive fibres (Fig. 2). The principal source of the AChE-positive fibres was from lesioned axons running in the alveus, cingulum and the supracallosal striae, belonging to the septo-hippocampal AChE-positive pathways¹² (Fig. 2c). There is now much evidence¹³⁻¹⁵ that this projection system is cholinergic. A contribution by peripheral AChE-positive parasympathetic fibres was only observed when the transplant reached all the way down to the interpeduncular fossa. In such transplants the parasympathetic fibres of the pial vessels¹⁶ were seen to have grown into the ventral part of the portal strip. In transplants that ended about 1.5 mm or more dorsal to the fossa no such ingrowth of peripheral cholinergic axons could be detected.

In vitro, the normal portal vein shows spontaneous rhythmic contractions with a frequency of about 1–3 min⁻¹ and a strength of about 500 dyne^{8,9}. Transmural stimulation elicits excitatory contractile responses that are blocked by PBZ and by previous sympathetic denervation^{9,17-19}. Exogenous NA and ACh are both excitatory on the myogenic activity of the portal vein, and these actions are totally blocked by α -adrenoreceptor blockers and atropine, respectively^{9,19}.

A total of 17 portal vein transplants were tested *in vitro* and of these 16 showed a good survival as judged by, first, their spontaneous myogenic activity, and, second, their contraction responses to NA and ACh administered to the bath. Some of the transplants had a regular, rhythmic spontaneous activity much resembling that of the intact portal vein (Fig. 1), but the contractions were weaker (about 20–110 dyne). The periods of activity were often more prolonged than normal, and occurred with a frequency of 0.5–2 min⁻¹. The remaining preparations had a more disordered spontaneous activity, without clear-cut periods of rest. Most of the specimens retained their spontaneous activity throughout the experiment (usually 2–2.5 h). Field stimulation at 6 to 10 Hz evoked excitatory contractile responses in 11 of the spontaneously active portal transplants. Optimally, the stimulation-induced contractions were of the same magnitude as those elicited by 10⁻⁶ M NA or ACh in the bath (Fig. 1).

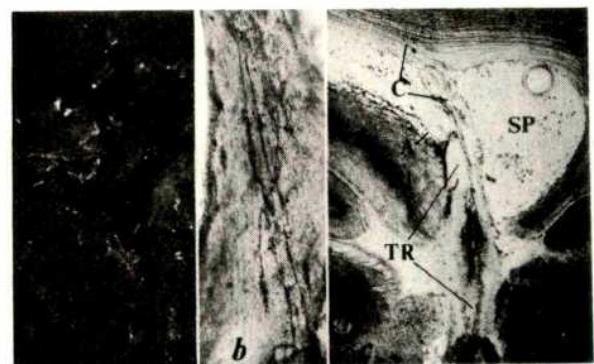
PBZ was used as the first blocker in 8 of the 11 preparations. The response to field stimulation was abolished in two, was reduced in four and remained virtually unaffected in two muscles of this group. Atropine was given to four of the six muscles with PBZ-resistant responses and in all

these the effect of field stimulation was completely abolished (Fig. 1). Three muscles received atropine as the first blocker. The response to field stimulation was abolished in one and remained in two of these preparations; these atropine-resistant responses were eliminated after PBZ. The results thus show that all responses to field stimulation could be abolished by PBZ and atropine, individually or in combination, indicating functional innervation of the muscles by adrenergic and/or cholinergic fibres. It may be pointed out that PBZ and atropine had no effect on the spontaneous myogenic activity but abolished specifically the responses to NA (10⁻⁶ M) and ACh (10⁻⁶ M), respectively. The efficiency of the receptor blockade was always tested by application of NA and ACh in these concentrations.

The fluorescence microscopical analysis of the *in vitro* stimulated transplants showed a good correlation between the PBZ-sensitive contractile responses to field stimulation and the presence of NA-containing fibres in the transplant. In the transplants showing no, or only very weak, spontaneous activities there occurred areas of necrosis, bleeding, or scar formation, and they were often poorly reinnervated from the brain.

The combined histochemical and physiological data provide strong evidence for functional neuromuscular connections being formed by the regenerated central adrenergic

Fig. 2 Reinnervation of portal vein transplants by central adrenergic and AChE-positive fibres, 25–30 d survival. *a*, Regenerated central NA-containing fibres in the muscle layer of the portal vein transplant, as seen in a transverse section of the transplant *in situ* (Falck–Hillarp method; $\times 75$); *b*, regenerated central AChE-positive fibres in a squash preparation of the isolated transplant ($\times 75$); *c*, sagittal section through the transplant in its position in the hippocampus (Hi) and the dorsal diencephalon (Di). AChE-positive fibres are seen to grow into the transplant (TR) from the alveus (A) and the cingulum (C). SP denotes the splenium of the corpus callosum. Rostral is to the right ($\times 16$).



and cholinergic axonal terminal networks in the transplanted portal strips. It is well established^{9,19,20} that transmural stimulation of the type used in the present study selectively excites the nerves in the tissue, and, therefore that the contractile responses are neuronally mediated. The effects of the receptor blockers observed in this study are consistent with this view and support the conclusion that the responses registered in the portal CNS transplants were due to the release of NA and ACh from the regenerated central fibres, acting on the smooth muscle receptors.

The adrenergic reinnervation was primarily accomplished by the locus coeruleus neurones. The characteristics of the neurotransmission restituted by the central adrenergic fibres in the transplant were quite similar to those of the normal portal vein^{17,18} or those of the portal vein reinnervated by peripheral sympathetic fibres²¹. Moreover, the efficacy of the central reinnervation was high in terms of the magnitude of the postsynaptic responses to neural stimulation. In fact, in the successful specimens they were as large as those evoked by a high concentration of exogenous NA.

Although it was not possible to remove surgically the intracranial parasympathetic innervation—as was done with the sympathetic one—it seems clear that at least in the more dorsally situated transplants the cholinergic neuroeffector responses were exclusively, or almost exclusively, mediated by regenerated central fibres, originating primarily from lesioned axons of the septo-hippocampal pathways. Interestingly, such cholinergic responses (remaining after α -adrenoreceptor blockade and being abolished by atropine) have not been detected in the normal rat portal vein although muscarinic cholinergic receptors are present^{9,17,18}. It therefore seems possible that in its location in the brain normally non-innervated cholinergic receptors will become reinnervated by the regenerating central cholinergic neurones, giving rise to a heterologous innervation similar to that described after cholinergic reinnervation of the cat nictitating membrane²²⁻²⁴.

The very limited restoration of function in adult mammals after brain damage is often explained by an insufficient regenerative capacity of central neurones and by their inability to re-establish functional connections^{25,26}. An important implication of the current studies on the reinnervation of peripheral transplants in the CNS would be that—when given the same growth conditions as in the peripheral nervous system—at least some neurone systems in the mammalian CNS will show an entirely adequate regenerative capacity resulting in a restitution of normal neurotransmission in the denervated tissue.

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Differentiation of opiate agonist and antagonist receptor binding by protein modifying reagents

OPIATE antagonists differ from opiates only by the replacement of the N-methyl substituent with an N-allyl, N-cyclopropylmethyl or related group. Antagonists can reverse the pharmacological effects of opiates and so are important in the treatment of opiate overdose. Drugs that combine agonist and antagonist activities have potential as relatively nonaddicting analgesics. Although their mutual pharmacological antagonism indicates that they act at the same binding site, pharmacological discrepancies have prompted speculation that agonist and antagonist binding sites are separate^{1,2}. Biochemical study of receptor binding³⁻¹⁶ has shown that although opiate agonists and antagonists compete for the same receptor^{3,4}, there are differences in the way they interact with them^{7,10}. Physiological concentrations of sodium enhance the binding of ³H-antagonists but reduce that of ³H-agonists. Sodium diminishes the ability of pure agonists to inhibit binding of ³H-naloxone to the receptor, but has little influence on inhibitory effects of pure antagonists and intermediate effects on those of combination agonist-antagonist drugs. The molecular basis for these differences, however, is unclear. As a first step in this direction we now report that binding of opiate receptors is much more sensitive to degradation by protein reagents which are known to modify sulphhydryl groups than is binding by opiate antagonists. This suggests that distinct binding sites exist for agonists and antagonists, although they may both be on the same receptor.

Brains, minus cerebella, from male Sprague-Dawley rats (180-220 g) were homogenised in standard buffer (50 mM Tris-HCl, pH 7.7 at 25°C) and centrifuged at 49,500g for 15 min; the pellets were resuspended in 100 volumes of standard buffer, incubated at 37°C for 30 min, recentrifuged and resuspended in 100 volumes of standard buffer. Incubation at 37°C increases opiate receptor binding about 80% (G.W.P. and S.H.S., in preparation). The homogenates were then treated with the appropriate reagent, washed by centrifugation at 49,500g for 15 min and assayed with 100 mM NaCl and the appropriate ³H-opiate at 25°C for 30 min in the presence and absence of 1 μ M levallorphan, unless otherwise stated. Samples were filtered and counted as before^{5,8}. All results are the mean of triplicate determinations and are expressed as specific opiate receptor binding, representing total binding minus binding in the presence of 1 μ M levallorphan.

Treatment of rat brain membrane preparations with iodoacetamide (20 mM) markedly reduced the specific receptor binding of the ³H-opiate agonists oxymorphone, levorphanol and dihydromorphone but not of the ³H-opiate antagonists naloxone and levallorphan (Table 1). To ensure that these effects were not due to interactions of iodoac-

Table 1 Effect of iodoacetamide on receptor binding of ^3H -opiate agonists and antagonists

Opiate	Stereospecific opiate binding (c.p.m.)		
	Control	Iodoacetamide Treated	% Change
Antagonists			
^3H -naloxone	1,040	1,092	+ 5
^3H -levallorphan	1,551	1,672	+ 8
Agonists			
^3H -oxymorphone	719	401	-44
^3H -levorphanol	1,288	827	-36
^3H -dihydromorphone	1,871	878	-53

Rat brains were homogenised in 20 volumes of standard Tris buffer and centrifuged at 50,000g for 15 min. The pellet was resuspended in 100 volumes of standard Tris buffer and equal volumes were incubated in the presence and absence of 20 mM iodoacetamide for 20 min at 25°C. The homogenates were then centrifuged as before, resuspended in their original volumes and assayed with either (+)-3-hydroxy-N-allylmorphinan or (-)-3-hydroxy-N-allylmorphinan (levallorphan) at 200 nM and the appropriate ^3H -opiate. Samples were filtered and counted as described in the text. The following concentrations of ^3H -opiates were used: 1.7 nM ^3H -naloxone; 2.8 nM ^3H -levallorphan; 3.7 nM ^3H -oxymorphone; 4 nM ^3H -levorphanol, and 0.7 nM ^3H -dihydromorphone.

tamide with ^3H -drugs themselves, the experiments were performed with membrane preparations presumably washed free of iodoacetamide. To explore the possibility that residual iodoacetamide might have reacted with the labelled ligands, thin-layer chromatograms in three solvent systems were prepared for all five ^3H -opiates after incubation with 100 mM iodoacetamide for 20 min at 25°C. Under these conditions there were no detectable changes in the relative fronts of any of the ^3H -drugs. Treatment with iodoacetamide did not alter the amount of radioactivity at the origin as would be expected for the products of an iodoacetamide-opiate reaction. Moreover, if the effects of iodoacetamide were due to reaction of the ^3H -opiates with residual reagent, an extra wash should have removed more iodoacetamide and lessened the inhibition of binding. Membranes subjected to a second centrifugation and resuspension showed no lessening of inhibition. Thus, the effects of iodoacetamide are irreversible and not due to chemical modification of the labelled ligands.

To examine the specificity and sensitivity of the differential degradation of agonist and antagonist binding, we compared the effects of various concentrations of iodoacetamide, mercuriacetate, iodoacetic acid, *p*-aminophenylmercuric acetate, *p*-chloromercuribenzoate and *N*-ethylmaleimide on opiate receptor binding of ^3H -naloxone and ^3H -dihydromorphone (Table 2). At 5 mM, iodoacetamide maximally inhibited binding of ^3H -dihydromorphone by about 90%; in other experiments 0.1 mM iodoacetamide lowered it by 25%. By contrast, binding of ^3H -naloxone showed little sensitivity to iodoacetamide between 5 mM and 100 mM. *N*-ethylmaleimide similarly discriminated between binding of ^3H -naloxone and ^3H -dihydromorphone. Concentrations of 0.1 mM to 3 mM *N*-ethylmaleimide maximally inhibited ^3H -dihydromorphone binding 90–100% and as little as 10 μM *N*-ethylmaleimide lowered it by 66%. Only a 30% decrease in ^3H -naloxone binding could be detected at 0.3 mM *N*-ethylmaleimide with 35% and 60% reductions in binding at 1 and 3 mM *N*-ethylmaleimide respectively. *p*-Chloromercuribenzoate also discriminated between agonist and antagonist binding. While 20 μM *p*-chloromercuribenzoate lowered ^3H -dihydromorphone binding by about 85%, it decreased that of ^3H -naloxone by only 45%. Differential effects on the binding of agonists and antagonists were also found for *p*-aminophenylmercuric acetate and mercuriacetate, although their effects were not as dramatic. There was a clear-cut separation of the effects of *p*-aminophenylmercuric acetate at 20 μM on binding of ^3H -naloxone and ^3H -dihydromorphone with a 75% decrease in ^3H -dihydro-

morphine binding and only a 45% decrease in ^3H -naloxone binding.

Unlike the other reagents, iodoacetic acid at 1–25 mM did not differentiate between agonists and antagonists, having little effect on the binding of either ^3H -dihydromorphine or ^3H -naloxone.

All the reagents which inhibit opiate receptor binding as described here are known to affect sulphhydryl groups, but can affect other amino acid residues^{17–23}, and so we investigated whether their action derives from an effect on sulphhydryl groups. Brain homogenates were incubated with 10 μM or 50 μM *p*-aminophenylmercuric acetate for 10 min at 25°C and centrifuged. The pellets were resuspended in the original volume of buffer, divided into aliquots and reacted with β -mercaptoethanol (10 mM and 20 mM for aliquots treated 10 μM and 50 μM *p*-aminophenylmercuric acetate, respectively) or water. Samples were again centrifuged and the pellets resuspended in their original volume of buffer and assayed with ^3H -dihydromorphine. The binding was abolished in samples treated only with 10 μM and 50 μM *p*-aminophenylmercuric acetate. In samples exposed to 10 and 20 mM β -mercaptoethanol after the *p*-aminophenylmercuric acetate (10 and 50 μM), however, there was only a 45% and 65% reduction in binding. The ability of a sulphhydryl-containing compound to reverse the lowering of opiate receptor binding suggests that *p*-aminophenylmercuric acetate decreases binding of ^3H -dihydromorphine by an action on sulphhydryl groups associated with the opiate receptor.

In preliminary studies to determine whether protein reagents modify the opiate binding site itself, we attempted to protect the binding site by preincubating with opiates before treatment with 10 μM iodoacetamide. The binding

Table 2 Effect of protein modifying reagents on receptor binding of ^3H -naloxone and ^3H -dihydromorphine

Reagent	^3H -Naloxone binding (% control)	^3H -Dihydromorphine binding (% control)
Iodoacetamide		
5 mM	86	9
25 mM	77	15
50 mM	80	16
100 mM	78	0
N-ethylmaleimide		
0.1 mM	85	7
0.3 mM	71	9
1.0 mM	65	16
3.0 mM	40	3
p-Aminophenylmercuric acetate		
10 μM	83	71
20 μM	54	26
40 μM	10	6
p-Chloromercuribenzoate		
20 μM	53	16
40 μM	34	13
Iodoacetate		
1 mM	100	90
3 mM	87	81
5 mM	98	89
25 mM	86	80
Mercuriacetate		
10 μM	79	42
100 μM	39	2

Rat brains were homogenised and prepared as described in the text. All reagents were dissolved in water immediately before use except for *p*-aminophenylmercuric acetate, mercuriacetate and *p*-chloromercuribenzoate, which were dissolved in dimethylsulphoxide. Dimethylsulphoxide in the concentrations used has no effect on binding. ^3H -naloxone was present at 1 nM and ^3H -dihydromorphine at 0.6 nM. All binding assays were performed in the presence of 100 mM NaCl. All experiments were replicated at least twice. Each value is the mean of triplicate determinations which varied less than 8%.

site for ^3H -dihydromorphine can be protected from the influence of iodoacetamide by preincubation with naloxone, oxymorphone, morphine and nalorphine at $0.2\ \mu\text{M}$ (our work in preparation).

Our findings thus demonstrate chemical differences in binding sites on the opiate receptor for agonists and antagonists. Sulphydryl groups seem to be important components for differentiating the interactions of opiate agonists and antagonists with the opiate receptor.

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Requirements for bursting pacemaker potential activity in molluscan neurones

THE mechanisms underlying endogenous rhythmical electrical activity of some molluscan neurones are not completely understood. Their membranes have several significant properties: (1) rapidly inactivating Na^+ and Ca^{2+} conductances associated with spikes¹⁻³; (2) voltage-dependent K^+ conductances (G_K) (delayed^{1,6} and anomalous rectification^{3,7}); and (3) a negative slope region (NSR) in the steady-state, voltage clamp current-voltage (I - V) curve^{8,9}. Although (1) is unnecessary for bursting pacemaker potentials (BPPs)⁴ the relationship of (2) and (3) to the generation of BPPs has not been elucidated completely. We present here evidence that the NSR of the I - V curve is due to a voltage-dependent Na^+ conductance (G_{Na}) which inactivates incompletely and that BPPs depend on this conductance coupled to cyclical changes in G_K .

In a typical experiment fused ganglia were isolated from the land snail (*Otala lactea*) or the sea hare (*Aplysia californica*), fixed in a chamber and perfused with saline (in mM, for *Aplysia*: 500 NaCl, 10 KCl, 50 MgCl_2 , 10 CaCl_2 ,

and 10 Tris-Cl, pH 7.8; for *Otala*: 100 NaCl, 4 KCl, 10 CaCl_2 , 5 MgCl_2 , 5 Tris-Cl, pH 7.8) at 20-21° C. Cell 11 of *Otala*⁵ or R15 of *Aplysia*² which normally generate BPPs were penetrated with two microelectrodes (3 M KCl or 7.5 M CsCl). Conventional methods were used to record the cell's electrical activity and voltage clamp its membrane potential. One microelectrode monitored V_m while the other carried current. The settling time of the system was about 1 ms for voltage steps up to 50 mV. Clamping current was measured by an operational amplifier that held the bath at ground.

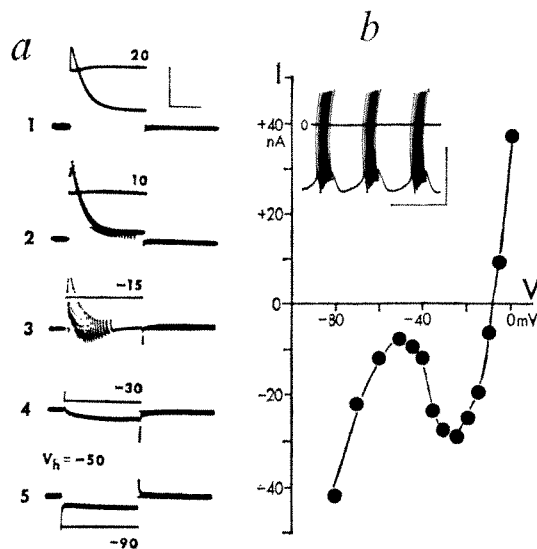


Fig. 1 Data from cell R15 of *Aplysia californica*. *a*, From voltage clamp. Voltage (thin lines) and current (thick lines) traces for 5-s pulse commands from holding potential (V_{holding} , V_h) of -50 mV to potentials indicated by numbers adjacent to voltage trace. Traces begin with voltage and current traces superimposed. Positive-going potentials and (outward) currents are displayed upward. Calibration: 45 mV for voltage traces, 500 nA (trace 1), 200 nA (trace 2), 100 nA (trace 3) and 50 nA (traces 4 and 5) for current traces; 2.5 s. See text for details. *b*, I - V curve from voltage clamp data shown in (*a*) (see text for details). Inset shows spontaneous BPP oscillations in the unclamped cell. Calibrations 50 mV, 25 s. In this and subsequent figures zero membrane potential is indicated by a horizontal line marked 0.

The steady-state current recorded from cells generating BPPs under voltage clamp are always inward (I_m) between -60 and -30 mV, the normal BPP range^{8,9}. The membrane was clamped between -60 and -30 mV to a potential which required the least holding I_m . Voltage step commands were applied (4-5 s) and the currents required to clamp the membrane at various potentials recorded. Four currents were identified: (1) an inward somatic spike current (not shown due to the slow time base); (2) a slowly decaying outward K^+ current (delayed rectification) for large depolarising steps (Fig. 1*a*, traces 1-3), (3) a voltage-dependent increase in I_m for steps between -45 and -30 mV, (trace 4) and (4) a time-dependent increase in inward current during large hyperpolarising commands (anomalous rectification) (trace 5). There were also rapid biphasic currents (traces 2 and 3) representing spikes in unclamped portions of the cell's axon.

I - V curves were constructed by measuring the minimum, slow changing current (least positive or most negative) during each voltage step at times greater than 1 s after the change in V_m and plotting this plus the holding current against the command voltage (Fig. 1*b*). Inward spike currents were excluded. Sometimes the slowly changing current was partially obscured by axon spike currents (for example, trace 3, where the minimum current was estimated at 2 s during the command step). The I - V curves were N-shaped (Fig. 1*b*) and the net, quasi-steady state current of cells generating BPPs rarely, if ever, became outward at

a V_m more negative than -30 mV. Qualitatively similar I - V curves were obtained in all cells generating BPPs. I_{in} from cell 11 was small but could be increased by replacing $[Ca^{2+}]_o$ and by iontophoresing Cs^+ ions intracellularly. This allowed quantitative analysis of the I_{in} and NSR.

The ionic mechanisms underlying the voltage-dependent I_{in} were assessed by stepwise replacement of most of Na^+ with equimolar amounts of $Tris^+$, Li^+ or sucrose. Reductions of $[Na^+]_o$ decreased the size of the I_{in} evoked at a given V_m (-30 mV in Fig. 2b) and of the NSR (Fig. 2c). All substitutions for $[Na^+]_o$ gave similar results (Fig. 2d): I_{in} at a given V_m varied linearly with $[Na^+]_o$. Reducing either the $[Cl^-]_o$ with SO_4^{2-} or methane sulphonate anions or the $[K^+]_o$ did not alter the I_{in} . These results suggest that the increased I_{in} evoked over the -50 to -30 mV V_m range is a Na current (I_{Na}) due primarily to a voltage-dependent Na^+ conductance (G_{Na}). This G_{Na} activates rapidly with depolarisation since the I_{in} develops quickly. The early decline in the I_{in} may represent some inactivation of G_{Na} but the persistence of I_{in} for the duration of a 5 s pulse indicates that G_{Na} does not inactivate completely. On returning V_m to the holding V_m , the inward tail currents relax within seconds, suggesting that G_{Na} inactivates quickly with hyperpolarisation.

The I - V properties of this persistent G_{Na} were evaluated

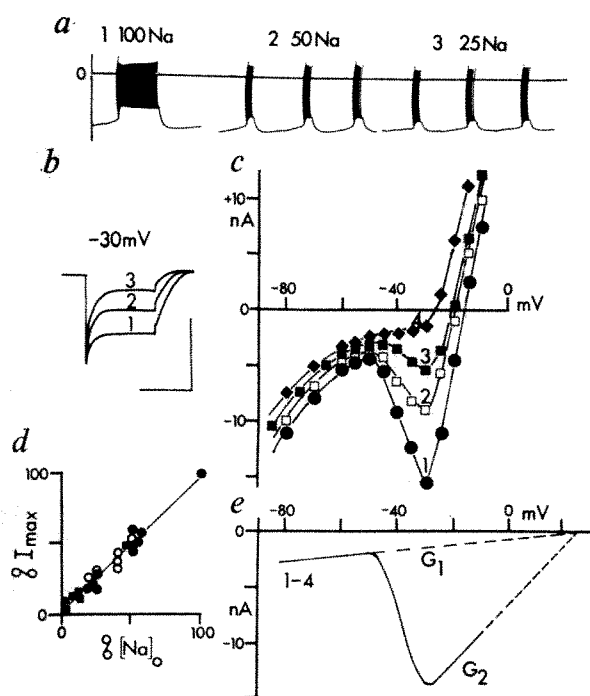


Fig. 2 Effects of altering $[Na^+]_o$ on membrane properties of BPP neurones. Data in (a-c) and (e) from cell 11 of *Otala lactea*. d contains data from both cell 11 and cell R15. Cs^+ ions were iontophoresed intracellularly by means of a microelectrode in cell 11 before measurements. In (a-c) data labelled 1 (or \bullet), 2 (or \square) and 3 (or \blacksquare) were obtained when the $[Na^+]_o$ was, respectively, 100, 50 and 25 mM (Na^+ replaced with $Tris^+$). Other extracellular ions were kept constant (10 mM $SrCl_2$, 4 mM KCl). a, Effect of reducing $[Na^+]_o$ on the BPP; b, line traces of voltage clamp currents recorded in pulsing the membrane from holding of -50 mV to -30 mV for 5 s in different $[Na^+]_o$ s; c, plots of voltage-clamp I - V curves. The curve linked by diamonds was taken in 25 mM $NaCl$, 80 mM $SrCl_2$, 4 mM KCl and 5 mM $Tris-Cl$. V_h always -50 mV; d, the clamp current, at -30 mV, as a percentage of current required to clamp the membrane at -30 mV in maximum $[Na^+]_o$ against the percentage of the maximum $[Na^+]_o$. $[Na^+]_o$ replacements were Li^+ (\blacksquare), $Tris^+$ (\bullet) and sucrose (\circ). e, Curve \bullet minus curve Δ of (c), see text. The solid parts of the curve are from the data in (c). The dashed lines are straight-line extrapolations, see text. Calibrations: 90 mV, 25 s (a); 12.5 nA, 4 s (b).

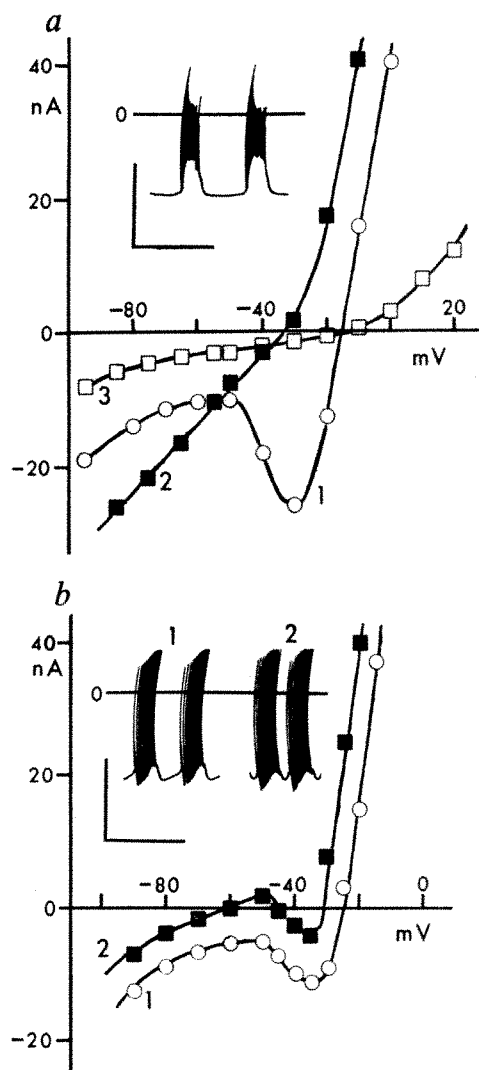


Fig. 3 Effects of extracellular ionic alteration on BPP activity and on membrane properties in cell 11 of *Otala lactea*. In all experiments extracellular $NaCl$ (100 mM) and $Tris-Cl$ (5 mM) were held constant. a, I - V (\circ) the medium also contained 10 mM $SrCl_2$ and 4 mM KCl and the cell had been injected with Cs^+ . Inset: BPPs under such conditions. \blacksquare , KCl was replaced with 8 mM $CsCl$; \square , the cell was returned to 4 mM KCl and the $SrCl_2$ replaced with 10 mM $CoCl_2$; V_h for all curves in a = -50 mV. b, For \circ the medium contained 10 mM $CaCl_2$ and 4 mM KCl , $V_h = -50$ mV. Inset 1: spontaneous BPP activity; \blacksquare the KCl was reduced to 1 mM and 5 mM $MgCl_2$ was added to the medium. The cell was silent at -60 mV but BPP activity could be elicited by injecting a steady 5 nA depolarising current through an intracellular microelectrode (inset 2). Calibration: 50 mV, 25 s.

by subtracting the I - V curve obtained when the persistent I_{Na} was assumed to be zero from that under control conditions (Fig. 2c, curve 1). The former conditions existed in a solution containing 25 mM $[Na^+]_o$ and 80 mM $[Sr^{2+}]_o$ which abolished the NSR of the curve without changing other measured currents (Fig. 2c, curve 4). The subtraction yields a curve (Fig. 2e) that indicates this persistent G_{Na} has voltage-independent and voltage-dependent components, which extrapolate to a reversal potential of about $+25$ mV, near the peak of the Na-dependent spike of this cell (Fig. 2a, part 1). These data suggest that the persistent I_{in} required to clamp the membrane at potentials in the NSR of the I - V curve is carried by Na^+ ions and that the NSR of the steady-state I - V curve is mainly due to a voltage-dependent, incompletely or non-inactivating G_{Na} . This persistent G_{Na} differs from the G_{Na} associated with spikes: (1) its thresh-

hold for activation is more negative (Fig. 1a); (2) inactivation is incomplete or absent (Figs 1a and 2b); (3) tetrodotoxin-sensitivity is absent⁴; (4) Li^+ cannot substitute for Na^+ in otherwise normal saline and (5) it is very temperature sensitive⁴.

Our data also indicate that the amplitude of the BPP (the difference between the trough and crest of a BPP) correlated with the magnitude of the persistent I_{Na} (Fig. 2a and d). Qualitative changes in these two variables (BPP, I_{Na}) resulted from other ionic manipulations: both were abolished by replacing $[\text{K}^+]_0$ with $[\text{Cs}^+]_0$ which markedly increased membrane conductance (Fig. 3a). When unclamped, the cell depolarised, fired repetitively and would not generate BPPs with hyperpolarising current (not shown). Both variables were also lost by replacing $[\text{Sr}^{2+}]_0$ with $[\text{Co}^{2+}]_0$ (Fig. 3a); again, BPP activity was not restored by extrinsic current. Others have shown correlations between the NSR and BPP activity when they were altered by temperature changes⁸ and pharmacologically⁹. While these data suggest that a persistent, voltage-dependent G_{Na} is required for BPP activity, it is not sufficient. Application of sufficient hyperpolarising or depolarising current can prevent BPPs^{3,4}, leading either to silence or repetitive firing. V_m behaviour changed similarly when the bathing solution was altered. When MgCl_2 was added and KCl was reduced the cell hyperpolarised to -60 mV and became silent; however, injection of a steady depolarisation current restored BPPs (Fig. 3b, inset 2). Conversely, after excessive Cs^+ injections, the cell shown in Fig. 3a depolarised to -15 mV and generated BPPs only with steady hyperpolarising current (Fig. 3a, inset). Under voltage clamp, both cells had a persistent G_{Na} and the V-axis intersected the I - V curves near the resting V_m of the unclamped membrane (-60 mV and -15 mV, respectively) (Fig. 3b, curve 2 and Fig. 3a, curve 1). Thus, these procedures resulted in a stable potential on a positive slope of the I - V curve and zero current was required to clamp V_m . Cells with spontaneous BPPs did not have a resting potential; the intersection

of the V-axis with their I - V curves was therefore, not a stable potential.

Another proposed requirement for BPP activity is a G_{K} with appropriate voltage- and time-dependencies^{3,12,13}. To study this contribution, V_m was clamped to -50 mV at different times during the BPP cycle and the resulting currents recorded (Fig. 4b, traces 1-4). (The record in Fig. 4a was not taken when the cell was clamped, but only indicates the times during the cycle when V_m was clamped.) The tail current following return of V_m to -50 mV from a 5 s command to -15 mV is also shown (Fig. 4b, trace 5). This tail current is most likely due to a G_{K} (refs 1, 6) for times longer than 5 s all currents decay exponentially with a time constant of about 16 s. If these are K^+ currents, the zero time intercepts of the curves in Fig. 4c (traces 1-4) reflect G_{K} at the onset of the clamp (values listed under $I_{\text{K}}(0)$ in Fig. 4b). G_{K} is minimal at the beginning of the bursting phase of the BPP (time 1), increases during the bursting period (times 2 and 3), becomes maximal at its end and decreases with time as the V_m slowly depolarises (times 4 to 1), as previously suggested^{3,12,13}.

In summary, two membrane properties are associated with BPP activity: (1) a G_{Na} with voltage-independent and voltage-dependent components which inactivates incompletely; (2) a voltage-dependent G_{K} with slow kinetics in the BPP range of V_m . Additional prerequisites include adequate membrane resistance and the absence of a steady-state electromotive force.

A possible mechanism of the interaction of these properties in producing spontaneous BPP activity is as follows. Let the V_m begin at its most hyperpolarised point; the slow decline in the voltage-dependent G_{K} which brought V_m to this point, along with the voltage-independent component of the persistent G_{Na} , depolarises V_m . When V_m reaches threshold for the voltage-dependent component of the persistent G_{Na} , it activates quickly and V_m depolarises rapidly. V_m remains depolarised from lack of inactivation of G_{Na} . This allows a burst of spikes and activates the voltage-dependent G_{K} which eventually generates sufficient hyperpolarising force to lead to the post-burst hyperpolarisation of V_m . This hyperpolarisation occurs rapidly since the voltage-dependent component of G_{Na} turns off quickly, and another BPP cycle begins. This description thus extends the hypothesis that pacemaker activity is due to a high resting G_{Na} and the cycling G_{K} . It also indicates the importance of the NSR in the I - V curve since this region is due to the voltage-dependent G_{Na} which inactivates incompletely.

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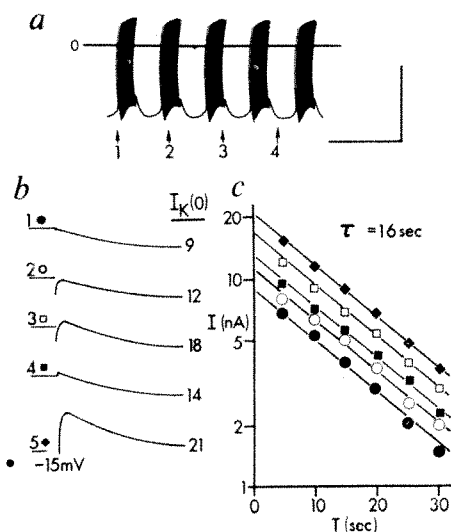
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Fig. 4 Currents recorded by voltage clamping at different phases of the spontaneous BPP cycle. a, Arrows indicate times during the BPP cycle when the cell was clamped to -50 mV; b, traces 1-4 are line drawings of the currents recorded at the times numbered in (a). Zero current indicated by the horizontal lines below the numbers in each trace. Trace 5: the tail current recorded when the cell was returned to V_h (-50 mV) from a 5-s step to -15 mV; c, semilog plot of current time for currents shown in (b). The slowly declining currents all fit an exponential with a time-constant of decay (τ) of about 16 s. Zero time was the onset of the clamp for 1-4 and the beginning of the tail-current in 5. Zero-time intercepts of each of these curves are listed under $I_{\text{K}}(0)$ in (b). Calibration: 60 mV, 30 s in (a); 60 nA, 30 s in (b).



Selection for diploid cells in suspension cultures of *Haplopappus gracilis*

CHANGES in the number and structure of chromosomes of plant cells cultured *in vitro* are of common occurrence¹, and cell populations devoid of diploid karyotype have been reported²⁻⁴. The chromosome number is known to become variable even in clones derived from single cells^{5,6}. In the case of *Picea glauca* Voss. ($n = 21$) callus cultures, a positive correlation between growth rate and chromosome number was observed⁷, which indicates a selection for polyploid cells. Only diploid cells were, however, observed in mitosis in suspension cultures of *Haplopappus gracilis* (Nutt.) Gray ($n = 2$) (ref. 2), *Medicago sativa* L. ($n = 16$) (ref. 8) and *Crepis capillaris* L. Wallr. ($n = 3$) (ref. 9). This could result from polyploid cells dividing less frequently than diploid cells, as diploid and polyploid cells differ physiologically. A strong selection for diploid cells was observed¹⁰ in *Vicia hajastana* Grossh. ($n = 5$) suspension cultures initiated from mature seeds. It therefore seemed likely that cell cultures of other plant species with a low chromosome number, $n = 7$ or less, would show a selection for diploid cells.

Callus cultures were initiated from hypocotyl segments of 3-d-old seedlings of *H. gracilis* on agar B5 medium¹¹ containing 4.5×10^{-6} M 2,4-dichlorophenoxyacetic acid (2,4-D). The calli were kept in a culture room at constant temperature (27–28°C) and light (2,000 lx). Subculturing was carried out every 3 weeks. After 122 d in culture, some cells were transferred to liquid B5 medium with 2,4-D at the same concentration. The suspension cultures were subcultured every 3 d, and were agitated by a gyratory shaker at 150 r.p.m. The cytological technique was essentially that of Singh *et al.*¹⁰.

An increase in the frequency of aneuploid cells was observed after 94 d in culture; a drastic decrease subsequently occurred. The frequency of diploid cells increased consistently whereas that of tetraploid cells steadily declined (Table 1). Anaphase analyses revealed a number of anomalies, such as bridges, fragments, unequal distribution of chromatids and laggards in low frequencies (1–3%). Chromosomes with altered mor-

phology were also observed at metaphase. These anomalies could have been induced by 2,4-D, or some other factor, present in the culture medium, and would lead to the production of aneuploid cells from diploid and polyploid cells. Furthermore, endoreduplication is likely to occur in plant cells cultured *in vitro*^{12,13}. The increase in the frequency of diploid cells under such a condition, therefore, clearly shows a strong selection for such cells. A similar selection for diploid cells was observed in suspension cultures of *H. ravenii* ($n = 4$), a closely related species, initiated from stem segments¹⁴.

The cytogenetic behaviour of *H. gracilis* cells has been investigated previously^{2,15-18}. In some studies, a systematic analysis of the changes in the frequencies of various karyotypes during the *in vitro* culture was not made^{2,16,17}, but where such an analysis was made^{15,18}, an increase in the frequency of polyploid cells was observed. Since agar media were used^{15,18}, these findings do not necessarily contradict the results reported here. An increase in the frequency of polyploid cells was observed when *H. gracilis* cells were cultured on agar B5 medium¹⁹. Only diploid cells were observed in a suspension culture of *H. gracilis*, which was interpreted to indicate a selection for diploid cells².

The distribution of the two chromosomes of *H. gracilis* in the aneuploid cells was non-random; chromosome I was present more often than expected. In fact, almost all of the cells with five chromosomes had an extra chromosome I. A similar non-random distribution of chromosomes has been observed in suspension cultures of *V. hajastana*¹⁰. Since both chromosome I and II seem to be equally susceptible to mitotic irregularities, it seems that cells possessing an extra chromosome I were at a selective advantage over those possessing an extra chromosome II.

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Table 1 Frequencies (%) of different cell types in two suspension cultures (A and B) of *H. gracilis*

Chromosome class	Days in culture				
	7	14	94	175	258
Hypodiploid (<4)					
Culture A	3.0	1.1	5.4	0.8	—
B	2.0	2.1	2.9	0.7	1.4
Mean	2.5	1.6	4.3	0.7	0.7
Diploid (2n = 4)					
Culture A	52.0	58.7	60.8	82.4	92.0
B	55.0	55.2	57.1	86.7	93.8
Mean	53.5	56.9	59.2	84.7	92.9
Hyperdiploid (5)					
Culture A	4.0	1.1	13.8	7.6	2.5
B	1.0	1.0	22.9	5.3	1.4
Mean	2.5	1.1	17.9	6.5	1.9
Hypotetraploid (6 and 7)					
Culture A	6.0	9.8	3.1	—	0.6
B	3.0	8.3	3.8	0.7	1.4
Mean	4.5	9.1	3.4	0.4	1.0
Tetraploid (4n = 8)					
Culture A	35.0	28.3	13.8	9.2	4.9
B	32.0	29.2	9.5	6.7	2.1
Mean	33.5	28.7	11.9	7.8	3.6
Hypertetraploid (>8 <16)					
Culture A	—	1.1	2.3	—	—
B	1.0	2.1	3.8	—	—
Mean	0.5	1.5	3.0	—	—
Octaploid (8n = 16)					
Culture A	2.0	—	0.8	—	—
B	4.0	2.1	—	—	—
Mean	3.0	1.1	0.4	—	—
No of cells observed					
Culture A	100	92	130	131	163
B	100	96	105	150	146
Total	200	188	235	281	309

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Lack of permeability of mouse placenta to maternal and foetal cells

IN man, foetal leukocytes circulate in the maternal blood in most pregnancies and are occasionally detected up to 1 yr after delivery¹⁻³. On the other hand, maternal white blood corpuscles have seldom been found in the foetal

circulation⁶⁻⁸. The reason for this paradoxical persistence of potentially histoincompatible cells in the mother's circulation is unknown. The fact that paternally-derived foetal HL-A antigens are difficult to demonstrate in cord blood samples⁹ suggests that the factors determining incompatibility in the foetal cells are either masked by blocking antibodies or are not expressed¹⁰.

In the mouse, information exists only on the passage of blood cells from mother to foetus¹¹⁻¹⁶. Both radioactive labelling¹¹⁻¹⁵, and cytogenetic markers^{12-14,16}, have been used to differentiate between foetal and maternal cells. The data are, however, inconsistent¹¹⁻¹⁶, probably resulting from the pitfalls of the methods used.

To overcome methodological difficulties, pregnancies resulting from laboratory mouse \times tobacco mouse matings were studied. The laboratory mouse has 40 acrocentric chromosomes, whereas the tobacco mouse has only 26 chromosomes, 14 of which are metacentric¹⁷. Since the hybrid has 33 chromosomes¹⁸, seven of which are metacentric, a mitosis from a foetus of such a cross can be distinguished without difficulty from a parental mitosis.

The passage of lymphocytes from foetus to mother was studied in phytohaemagglutinin (PHA) or leucoagglutinin (LA) cultures of blood from female laboratory mice mated with male tobacco mice. Blood samples were taken between the second and third weeks of pregnancy (in ten instances), or immediately after delivery (in five instances), and cultured as described¹⁹. Chromosome preparations were made by conventional methods²⁰ and stained with Giemsa; 100-1,000 mitoses were studied from each animal.

Transmission of blood cells in the opposite direction, from mother to foetus, was studied in identical matings. Ten newborn mice (two from each of five litters) were killed and chromosome preparations made from liver, spleen, bone marrow and thymus¹², and stained with Giemsa. A total of 10,000 mitotic cells was studied.

Table 1 Mitotic studies on cultures of lymphocytes from female laboratory mice mated with male tobacco mice

	No. of animals	No. of cells	No. of foetal cells
During pregnancy	10	4,995	0
After delivery	5	4,096	0
Total	15	9,091	0

PHA or LA cultures were carried out between weeks 2 and 3 of pregnancy in ten animals, and immediately after delivery in five animals.

The results of chromosome studies on maternal lymphocyte cultures are presented in Table 1. Foetal mitoses were never found in these cultures; neither was there any evidence of transmission of blood cells from mother to foetus.

The results of Tuffrey *et al.*^{12,13}, however, indicate transmission of maternal cells into the foetus. These workers used the T₆ chromosome marker in a syngeneic system to distinguish foetal mitoses from maternal mitoses and found maternal cells in up to 33% of mice which had developed in the uterus of foster mothers after transplantation at the blastocyst stage. Later, using the same marker, they found maternal cells in the offspring after normal pregnancies. Others^{14,16} using the T₆ marker, have been unable to confirm these data, and suggest that differences between mouse strains or misinterpretation of the T₆ chromosome are possible reasons for these discrepancies. The main limitation of these studies is that only mitoses, and not interphase cells, have been studied. Negative results might be due to inability of foetal and maternal cells crossing the mouse placenta to enter mitotic division in such experiments. We have also examined mitotic cells.

Radioactive labelling has been used to mark maternal blood cells in the circulation of the offspring^{11,15}. One group¹¹ was able to demonstrate transplacental passage of red blood corpuscles in a few pregnancies, and the other¹⁵

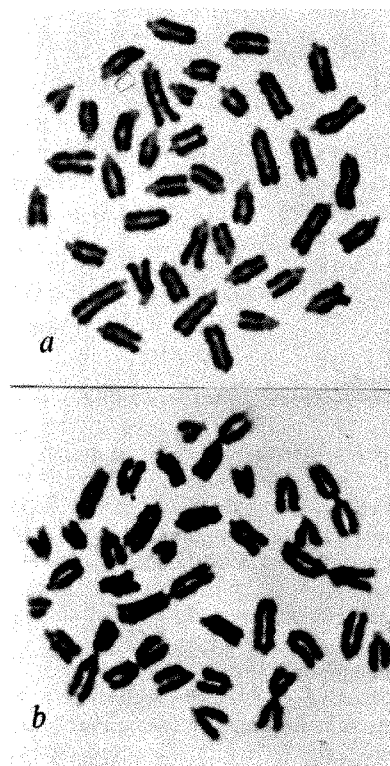


Fig. 1 *a*, Cultured lymphocyte of a laboratory mouse in mitosis. The cell has 40 acrocentric chromosomes. *b*, A mitosis from a chromosome preparation of liver from a laboratory mouse \times tobacco mouse hybrid. The cell has 33 chromosomes, seven of which are metacentric.

found maternal erythrocytes and leukocytes in the blood of most foetuses. The method is open to question, however, as passage of free isotope molecules across the placenta and uptake of these by foetal cells cannot be excluded.

The human placenta seems to be permeable to blood cells, and foreign cells invade the circulation of both the foetus and the mother often without causing any clinical symptoms²⁻⁸ and so it may seem surprising that this is not so in the mouse. Reasons may be that the placental structure of the two species differ; differences in the number of foetuses, time of gestation or in the validity of the methods used to study the phenomenon may also be responsible. In man, the methods seem to be adequate and the results convincing, but in the mouse suitable markers are lacking. One limitation is the lack of markers for mitotic studies, the other the failure to devise a method for distinguishing between foetal and maternal cells during interphase. We have overcome the first problem but not the second. We have not found any evidence for the transplacental passage of blood cells in the mouse, but the results must be regarded with caution until markers are found by which foetal and maternal cells can be distinguished from each other at interphase.

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Cytochalasin B, the blood platelet release reaction and cyclic GMP

CYTOCHALASIN B (CB) affects a wide range of cellular processes that appear to be contractile in nature¹. The molecular mechanisms responsible for these effects are uncertain, though it is known that CB interferes with microfilament function¹ and can block transport of glucose across cell membranes^{2,3}. Secretion by exocytosis has been shown to be inhibited by CB in some cell types⁴⁻⁸ but to be potentiated by CB in others⁹⁻¹². We describe here the effects of CB on the release reaction of blood platelets induced by collagen fibres, a process in which the contents of both amine storage granules (5-HT, ADP, ATP) and lysosomes are secreted¹³ and which leads to platelet aggregation mediated by the ADP released¹⁴. Although previous studies have shown that CB inhibits various platelet activities including clot retraction¹⁵⁻¹⁷ and platelet aggregation^{16,17}, we now find that lower concentrations of CB markedly potentiate both the secretory activities of the platelet and release-dependent aggregation. Because collagen-induced platelet aggregation is associated with an increase in intracellular cyclic GMP¹⁸, a compound which potentiates secretion by some cells^{19,20}, and

because it has been suggested that CB may stimulate leukotaxis²¹ by increasing cyclic GMP²¹, we have studied the relationship between the platelet release reaction and platelet cyclic GMP levels in the presence and absence of CB. The results show that CB does not potentiate the release reaction through any direct effect on cyclic GMP levels.

Experiments were performed either with human heparinised platelet-rich plasma (PRP) adjusted to contain 470,000 platelets μl^{-1} (ref. 22) or with a suspension of washed human platelets ($560,000 \mu\text{l}^{-1}$) suspended in Tyrode's solution containing 0.35% bovine serum albumin and apyrase²³. Platelet 5-HT was labelled by preincubating PRP with $1 \mu\text{M}$ ^{14}C -5-HT (ref. 22) for 1 h or washed platelets with $2 \mu\text{M}$ ^{14}C -5-HT for 20 min during the first wash. Samples (0.85 ml) of labelled PRP or washed platelet suspension were mixed with $2 \mu\text{l}$ dimethyl sulphoxide \pm CB and $48-128 \mu\text{l}$ 0.154 M NaCl and were incubated at 37°C for 10 min before addition of further 0.154 M NaCl \pm collagen to give a final volume of 1 ml. Samples were transferred to an apparatus for the turbidometric measurement of platelet aggregation (Payton Associates Ltd., Scarborough, Ontario) 0.5 min or 1 min before the last addition and then stirred continuously. Incubations were usually terminated by addition of 0.1 ml 0.077 M Na_3EDTA and centrifugation for 0.5 min at $12,000g$ (Eppendorf 3200). ^{14}C and enzyme activities were measured in the supernatants and in a corresponding volume of PRP or washed platelet suspension in which the platelets were lysed (see Table 1 for methods). Values in the supernatant from a control without CB or collagen were subtracted and the percentage release of platelet constituents calculated. CB did not affect the activities of the enzymes assayed. Bovine collagen (Sigma) was prepared for use by homogenisation in 0.154 M NaCl at 0°C (Polytron homogeniser); coarse fibres were removed by centrifugation ($100g$, 5 min). The collagen remaining in suspension was determined gravimetrically after high speed centrifugation, washing with water and drying at 110°C .

Preliminary experiments established that 0.2% dimethyl sulphoxide (the solvent for CB) did not affect the release reaction and that CB did not affect the uptake of ^{14}C -5-HT by platelets. Addition of CB without collagen did not cause

Table 1 Effects of CB on the release of platelet constituents induced by collagen fibres

Experiment 1	Additions		^{14}C -5-HT	Release of platelet constituents (%)			Lactate dehydrogenase
	Collagen ($\mu\text{g ml}^{-1}$)	CB ($\mu\text{g ml}^{-1}$)		β -Glucuronidase	β -N-Acetylglucosaminidase		
	0	5	<1	<1	<1		<1
		50	<1	<1	<1		<1
	1	0	1	<1	<1		<1
		1	14	<1	2		<1
		2	17	2	3		<1
		5	30	3	2		<1
		10	44	4	6		<1
		50	26	<1	3		2
	30	0	75	11	17		<1
		1	79	14	31		2
		2	81	21	35		<1
		5	81	21	35		1
		10	80	21	37		3
		50	75	12	35		3
Experiment 2	0	0.1	<1	<1	<1		<1
		10	<1	<1	<1		<1
	6.5	0	9	1	8		<1
		0.03	15	3	8		<1
		0.1	28	5	14		<1
		0.3	23	3	12		<1
		1	2	<1	2		<1
		10	1	<1	<1		<1

PRP was used in experiment 1 and washed platelets in experiment 2. Final concentrations of collagen and CB are given. Incubations were terminated 3 min after addition of collagen. ^{14}C was counted by liquid scintillation¹⁸. β -Glucuronidase and β -N-acetylglucosaminidase were assayed at 30°C in 50 mM citrate buffer, pH 4.5, containing 0.25% Triton X-100 and 4 mM *p*-nitrophenyl- β -D-glucuronide or *p*-nitrophenyl-N-acetyl- β -D-glucosaminide respectively. After 20 h or 2 h respectively these reactions were stopped with 9 volumes 0.02 N NaOH and the *p*-nitrophenol liberated was measured spectrophotometrically at 405 nm. Lactate dehydrogenase was assayed at 30°C in the presence of 0.1% Triton X-100 using a standard method²⁴.

Table 2 Effects of CB on the platelet ^3H -cyclic GMP levels observed in the presence of weak and strong release stimuli

Additions	Release of ^{14}C -5-HT (%)	^3H -Cyclic GMP d.p.m. per sample	2P
—	—	$1,297 \pm 105$	—
CB	<1	$1,436 \pm 44$	>0.2
Collagen (2 $\mu\text{g ml}^{-1}$)	1	$2,222 \pm 357$	—
Collagen (2 $\mu\text{g ml}^{-1}$) + CB	23	$4,761 \pm 465$	<0.02, >0.01
Collagen (14 $\mu\text{g ml}^{-1}$)	48	$4,214 \pm 160$	—
Collagen (14 $\mu\text{g ml}^{-1}$) + CB	62	$4,230 \pm 296$	>0.95
Collagen (136 $\mu\text{g ml}^{-1}$)	76	$5,519 \pm 786$	—
Collagen (136 $\mu\text{g ml}^{-1}$) + CB	78	$6,844 \pm 778$	>0.2
ADP (2 μM)	2	$4,555 \pm 261$	—
ADP (2 μM) + CB	1	$4,919 \pm 159$	>0.2

The final CB concentration was $5 \mu\text{g ml}^{-1}$ when present. Incubations were terminated 2 min after addition of the release stimulus (final concentration given). ^3H -Cyclic GMP values are means \pm s.e.m. from three identically treated samples of PRP. The significance of the effects of CB was determined by unpaired Student's *t* tests.

aggregation or induce the release of platelet constituents. But addition of $1\text{--}20 \mu\text{g CB ml}^{-1}$ to PRP before a threshold amount of collagen markedly increased the extent of platelet aggregation (Fig. 1). This was associated with a several-fold increase in release of ^{14}C -5-HT, suggesting that the enhanced aggregation was mediated by ADP released with the 5-HT. An almost identical potentiation of ^{14}C -5-HT release was observed whether CB was added with the collagen or up to 50 min before. The greatest increase in release was with $10 \mu\text{g CB ml}^{-1}$ in PRP (Fig. 1; Table 1, experiment 1). Despite these effects, CB delayed platelet aggregation and at concentrations greater than $20 \mu\text{g ml}^{-1}$ suppressed the change in platelet shape (upward deflection of

the recording) that normally precedes aggregation (Fig. 1). Higher CB concentrations ($> 40 \mu\text{g ml}^{-1}$) also delayed and inhibited the release reaction. With amounts of collagen that induced complete platelet aggregation only the inhibitory effects of CB on aggregation were seen in turbidometric recordings, even when release of ^{14}C -5-HT was potentiated. Collagen, particularly at high concentrations, also induced the release of the lysosomal enzymes, β -glucuronidase and β -N-acetylglucosaminidase, from platelets in PRP. In the presence of $10 \mu\text{g CB ml}^{-1}$ the amounts released were approximately doubled (Table 1). In no experiment were more than trace amounts of lactate dehydrogenase released, indicating that CB specifically potentiated the release reaction rather than causing a generalised leakage of cell constituents. Very similar results were obtained with washed platelets (Table 1, experiment 2), but the concentration of CB required for maximum potentiation of the release reaction was much lower ($0.1 \mu\text{g ml}^{-1}$). Above $1 \mu\text{g ml}^{-1}$, CB almost completely suppressed both the release of washed platelet constituents and aggregation.

Our finding that low concentrations of CB can potentiate the platelet release reaction suggests that under appropriate conditions other secretory processes reported to be inhibited by CB^{4-8} may be similarly potentiated. In two instances there is already evidence for this^{25,26}. The question arises whether the potentiation and inhibition of the release reaction by CB involve different mechanisms of action at the molecular level. Lin *et al.*²⁷ have reported that bovine platelets have high and low affinity binding sites for CB and that serum reduces binding to both. The dissociation constants of CB at these two classes of binding site in the presence and absence of serum correlate moderately well with the respective concentrations we required for potentiation or inhibition of the release reaction in the human PRP and washed platelet systems. Lin *et al.*²⁷ suggest that their high affinity sites may be associated with hexose transport and the low affinity sites with cellular contractility. Although CB does block glucose metabolism in platelets¹⁷, it seemed unlikely that this could potentiate the release reaction. We therefore investigated the possibility that cyclic GMP might mediate this action of CB.

To permit measurement of changes in platelet cyclic GMP by a prelabelling method¹⁸, $2 \mu\text{M } ^3\text{H}$ -guanine (4 Ci mmol^{-1}) was included with ^{14}C -5-HT in the preincubation step in some experiments with PRP. ^3H -Cyclic GMP was then isolated from incubations stopped by addition of $0.2 \text{ ml } 3 \text{ N HClO}_4$ with unlabelled cyclic GMP¹⁸. Collagen caused a concentration-dependent increase in platelet ^3H -cyclic GMP up to about four times the basal level (Table 2). The ^3H -cyclic GMP level increased rapidly after a short interval at approximately the same time as the most rapid release of ^{14}C -5-HT (Fig. 2). As release could not be measured instantaneously, we could not determine whether or not the increase in ^3H -cyclic GMP preceded the release reaction. After the initial rapid increase, the ^3H -cyclic GMP concentration rose more slowly for at least 3 min, despite cessation of release and some reuptake of

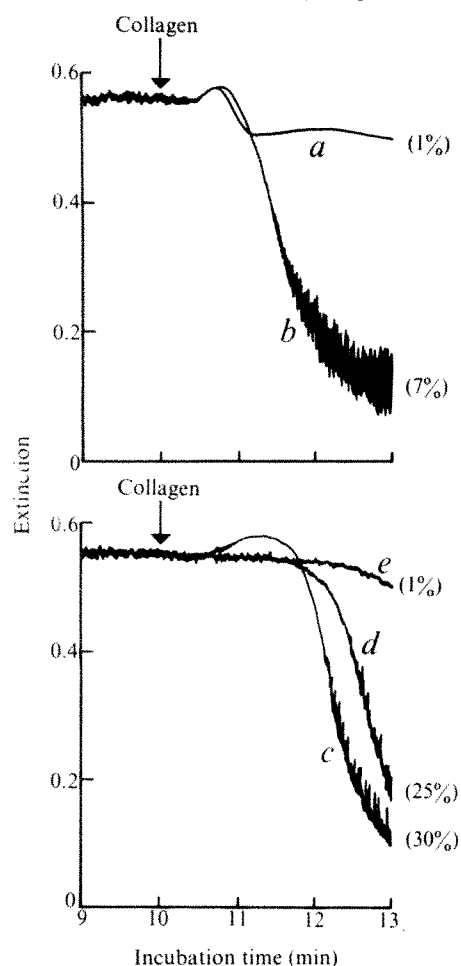


Fig. 1 Effects of CB on platelet aggregation and release of ^{14}C -5-HT induced by collagen. The amount of collagen added (final concentration $2 \mu\text{g ml}^{-1}$) was adjusted so as to cause only slight platelet aggregation in the absence of CB. Incubations were terminated 3 min after addition of collagen. Release of ^{14}C -5-HT (%) at this time is indicated in parentheses. Final CB concentrations ($\mu\text{g ml}^{-1}$) were: a, 0; b, 2; c, 10; d, 20; e, 40.

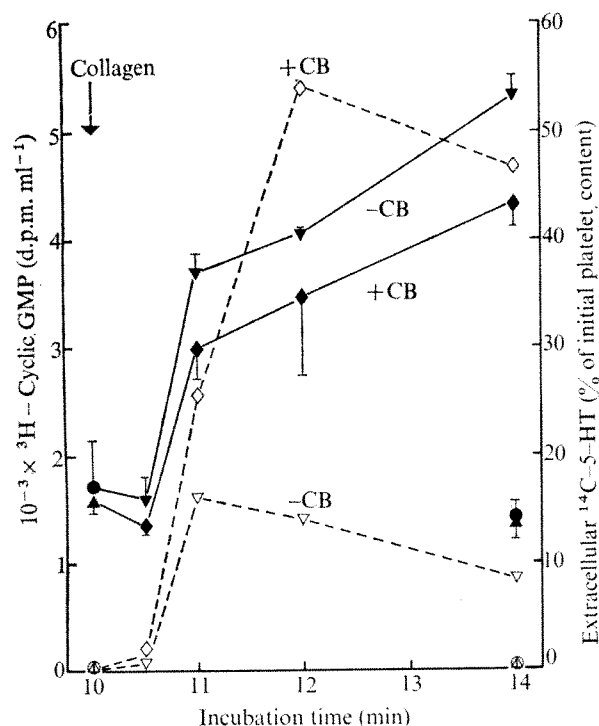


Fig. 2 Platelet ^3H -cyclic GMP levels during release of ^{14}C -5-HT induced by collagen in the presence and absence of CB. Final CB and collagen concentrations were 10 and $5.4 \mu\text{g ml}^{-1}$ respectively when present. ^3H -Cyclic GMP values are means \pm s.e.m. from three identically treated samples of PRP. Closed symbols, ^3H -cyclic GMP (d.p.m.); open symbols, ^{14}C -5-HT released (%). \circ , \bullet , Controls without CB or collagen; Δ , ∇ , CB alone; ∇ , Δ , collagen alone; \diamond , \diamond , CB and collagen.

^{14}C -5-HT. CB (5 or $10 \mu\text{g ml}^{-1}$) had no statistically significant effect on basal ^3H -cyclic GMP levels or on the increases caused by moderate or high doses of collagen, although it markedly potentiated the release reaction induced by the former (Fig. 2, Table 2). These findings indicate that cyclic GMP does not mediate the potentiation of release by CB. With threshold doses of collagen, however, CB did increase ^3H -cyclic GMP to a highly significant extent (Table 2). This can be explained by the observation that addition of ADP alone also increased ^3H -cyclic GMP (Table 2). Thus, potentiation of the release of ADP by CB probably provided an important extracellular stimulus for the formation of ^3H -cyclic GMP, when little collagen was present. That collagen itself at higher concentrations also stimulates formation of cyclic GMP is indicated by previous observations that inhibition of collagen-induced release and aggregation by aspirin does not block the associated increase in platelet ^3H -cyclic GMP¹⁸. Addition of ADP with or without CB did not cause release of any platelet ^{14}C -5-HT (Table 2). Thus, even if an increase in cyclic GMP is necessary for the release reaction to occur, other factors provided by collagen but not ADP in heparinised PRP are also required. The results indicate that increases in ^3H -cyclic GMP in human platelets may be a relatively nonspecific response to stimulation. Whether platelet aggregation, the release reaction or other platelet activities are facilitated in the presence of increased intracellular cyclic GMP levels remains to be established.

If the potentiation of the release reaction by CB is not mediated by cyclic GMP, what other possibilities exist? It is conceivable that a single class of membrane binding sites could both inhibit glucose transport and facilitate the membrane fusion required for exocytosis²⁸. These membrane sites could be on a protein, possibly platelet actin or myosin, elsewhere associated with the contractile apparatus of the platelet in such a way that their affinity for CB is reduced^{29,30}. Interaction of CB with these latter sites would then account for the inhibitory action of the compound on platelet activities.

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Changed surface glycoprotein as a marker of malignancy in human leukaemic cells

A STRUCTURAL difference in fucose-containing glycopeptides isolated from the surface of virally transformed fibroblasts has been described^{1,2}. Further studies revealed that this difference was caused by increased sialic acid content of the glycopeptides³. Previously we demonstrated⁴ that this phenomenon is not limited to the surface glycoprotein of virally transformed fibroblasts but also occurs in spontaneously chemically or virally transformed cells of fibroblastic, epithelioid and lymphoid morphologies maintained *in vitro*. Since similar results were obtained with mouse lymphosarcoma and rat hepatoma cells grown *in vivo* (ref. 5 and manuscript in preparation), we have extended our observations to human tumour biopsies. We now report that analogous changes occur in the surface glycopeptides of tumour cells obtained from peripheral blood of patients with active leukaemia or with leukaemic transformation of lymphosarcoma. In all cases peripheral blood from untreated patients containing at least 80% leukaemic cells in the white blood cell fraction, were used.

Normal resting and phytohaemagglutinin (PHA) stimulated peripheral blood lymphocytes served as controls. Blood lymphocytes from patients in various stages of infectious mononucleosis (IM) were used as an additional control for a non-malignant lymphoproliferative disease.

Defibrinated blood samples were run on a Ficoll-Isopaque gradient⁶ and lymphocytes or blasts were collected

Table 1 Chromatographic analysis of surface glycopeptides from various leukaemic cells of human origin

Patient	Child/adult	*Diagnosis	Recovered WBC mm ⁻³	†Relative elution profiles	
				minus NANase	plus NANase
1	A	ALL	20,000	+++	0
2	C	ALL	5,000	++	nd
3	A	CLL	40,000	+	0
4	A	CLL	27,000	+++	nd
5	A	AML	54,000	+++	0
6	C	AML	120,000	++	0
7	C	CML(ju)	10,000	++	+++
8	A	CML	60,000	+++	+++
9	A	LS	37,000	+	—
10	C	LS	80,000	+	nd
11	A	AML(pro)	3,500	++	—
12	C	AMMoL	107,000	++	0

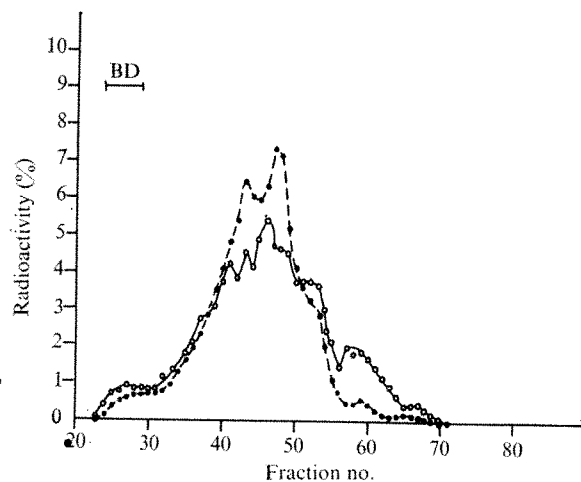
*ALL, acute lymphocytic leukaemia; CLL, chronic lymphocytic leukaemia; AML, acute myelocytic leukaemia; CML (ju), chronic myelocytic leukaemia (juvenile type); LS, lymphosarcoma; AML (pro), acute promyelocytic leukaemia; AMMoL, acute myelomonocytic leukaemia.

†The signs denote the number of 0.75 ml fractions that the malignant material eluted ahead (+) or after (—) the control material derived from normal lymphocytes (0, coinciding) as measured after elution of 50% of the radioactivity in the ascending limb of the profile. NANase, neuraminidase treatment; nd, not determined.

from the interphase, washed and counted (Tables 1 and 2: recovered white blood cells (WBC)). Enrichment in myelocytic leukaemic blasts by partial loss of differentiated cells is not excluded by this method. The washed cells were incubated in RPMI 1640 medium supplemented with 20% foetal calf serum and antibiotics in small Erlenmeyer flasks at a density of 4×10^6 cells ml⁻¹. The cells were differentially labelled by incubation for 2 or 3 d in the presence of ¹⁴C-fucose (0.25 μ Ci ml⁻¹) in the case of control lymphocytes or ³H-fucose (0.50 μ Ci ml⁻¹) in the case of tumour cells, IM cells or PHA-stimulated lymphocytes. PHA stimulation was carried out by the inclusion of the mitogen (50 μ g ml⁻¹) in the incubation medium.

Tumour cell numbers did not change during incubation and viability exceeded 90% as measured by trypan blue dye exclusion except in some cases of lymphosarcoma cells (patient 10, Table 1). After incubation, the cells were collected by centrifugation, washed and mildly trypsinised. The solubilised materials from control and tumour, IM or PHA-stimulated cell samples were mixed and exhaustively digested

Fig. 1 Elution profile of L-fucose-containing glycopeptides of pronase-digested trypsinates from differentially labelled PHA-stimulated (●) and resting normal (○) peripheral lymphocytes following filtration through Biogel P10–Sephadex G-50 fine (2:1). The radioactivity of the fractions is represented as percentages of total radioactivity eluted from the column. The column markers blue dextran (BD) and phenol red eluted from the column in fraction range 23–29 and 83–94 respectively.



with Pronase, followed by dialysis and concentration by lyophilisation. The resulting glycopeptides were filtrated over Biogel P10(200–400 mesh)–Sephadex G-50 fine (2:1) columns (1 × 100 cm).

Full experimental details including conditions of neuraminidase treatment of the glycopeptides have been published elsewhere⁴.

Cochromatography of the Pronase-digested, fucose-labelled glycopeptides obtained from resting and PHA-stimulated peripheral lymphocytes showed that the ascending limbs of the elution profiles coincided (Fig. 1). The main peak was increased, but not displaced, in the case of stimulated cells at the expense of lower molecular weight material, similar to the difference observed in log and plateau phase fibroblasts⁷.

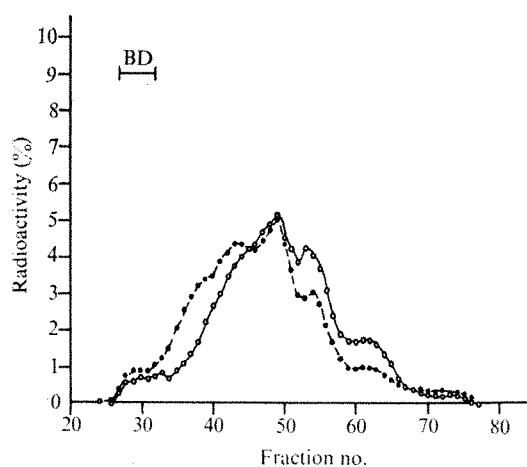


Fig. 2 Elution profile of L-fucose-containing glycopeptides of Pronase-digested trypsinates from differentially labelled peripheral leukaemic cells from patient 4 (Table 1) with CLL (●) and from normal peripheral lymphocytes (○) following filtration through Biogel P10–Sephadex G-50 fine (2:1). The radioactivity of the fractions is represented as percentages of total radioactivity eluted from the column. The column markers blue dextran (BD) and phenol red eluted from the column in fraction range 26–31 and 87–96 respectively.

The results of cochromatography of malignant and normal lymphocyte glycopeptides are summarised in Table 1. In all experiments, including a permanent line of Burkitt's lymphoma (not illustrated), the fucose-labelled glycopeptides of the malignant cells eluted ahead of the controls. The degree of this horizontal displacement was estimated at half-maximal height of the ascending limb of the profiles and expressed as the number of fractions eluting ahead of the control. The difference is represented in Table 1 by the corresponding number of (+) signs. Differences among the elution profiles of individual malignant cell samples were

Table 2 Chromatographic analysis of surface glycopeptides from lymphocytes of patients with infectious mononucleosis

Patient	Child/adult	Recovered* WBC mm ⁻³	Atypical lymphocytes (%)	Paul Bunnell* titre	Relative† elution profiles
1	C	16,500	34	1:2048	0
2	A	14,000	33	1:2048	—
3	C	11,000	NR	1:32	—
4	A	5,000	>30	1:1024	0
5	C	5,000	NR	NR	—
6	A	5,000	3	Positive	—
7	C	3,000	36	1:512	0
8	A	2,500	35	1:1024	0
9	C	2,000	>30	1:32	0
10	C	1,500	35	1:512	0

*Normal values: Recovered WBC 1,000–2,000 mm⁻³; Paul Bunnell titre <1:32.

†See Table 1.

||NR, Not reported.

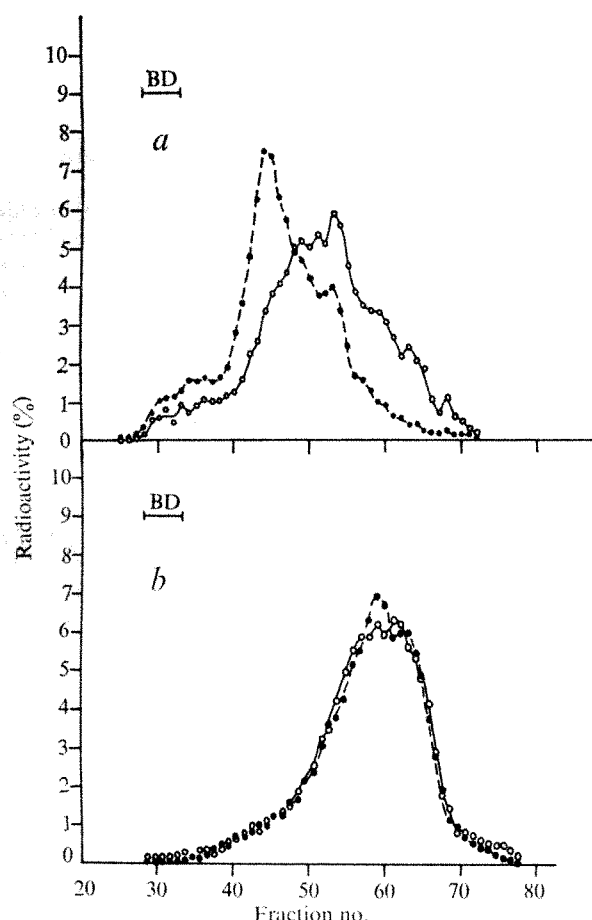


Fig. 3 Elution profiles of L-fucose-containing glycopeptides of Pronase-digested trypsinates from differentially labelled peripheral leukaemic cells from patient 5 (Table 1) with AML (●) and from normal peripheral lymphocytes (○) before (a) and after (b) neuraminidase treatment following filtration through Biogel P10–Sephadex G-50 fine (2:1). The radioactivity of the fractions is represented as percentages of total radioactivity eluted from the column. The column markers blue dextran (BD) and phenol red eluted from the column in fraction range 28–33 and 99–107 respectively.

noted not only in the degree of horizontal displacement but also in the overall shape of the profile, as similarly observed earlier for various transformed cell lines *in vitro*⁶. Thus, the glycopeptides from chronic lymphocytic leukaemia (CLL) cells simply eluted ahead (Fig. 2), whereas AML (acute myelocytic leukaemia) glycopeptides showed a discrete peak ahead of the main peak of the control (Fig. 3a). It remains to be established whether details of elution profiles correlate with the type of the disease.

The glycopeptides of nine cell samples, each mixed with control glycopeptides, were incubated with *Vibrio cholerae* neuraminidase (Behringer, 50 U ml⁻¹, for 120 min) before cochromatography. In seven cases, the enzyme treatment eliminated the difference in the elution profiles between tumour and normal samples (0, Table 1; Fig. 3b) or caused tumour glycopeptides to elute after the controls by one fraction (—, Table 1). From similar results with cultured normal and transformed cells it has been concluded previously that an increase of neuraminidase-sensitive sialic acid in the transformed-cell glycopeptides is responsible for their being eluted ahead of the corresponding normal material^{3,4}. Apparently the same molecular change occurs in the glycopeptides obtained from the human leukaemic cells studied here.

It may be significant that only in the two cases of chronic myelocytic leukaemia (CML) cells no 'normalisation' of the elution profiles by neuraminidase treatment was obtained. Unpublished results with certain experimental

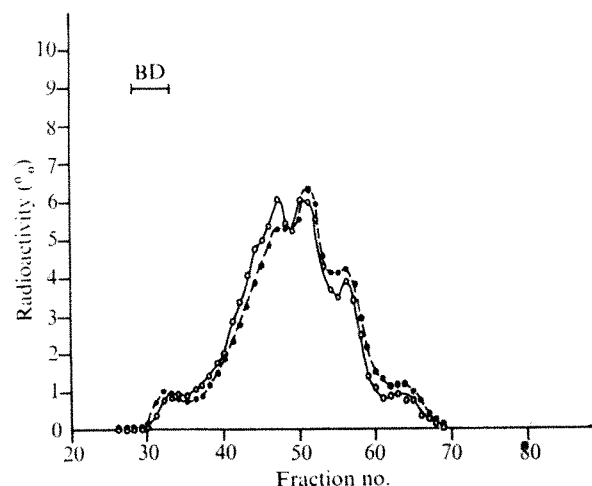
tumours suggest that in such cases either neuraminidase-insensitive sialic acid or an additional anionic (sulphated or phosphorylated) sugar residue could be involved. Further experiments are needed to establish whether CML cells may be distinguished from other leukaemia cases by this criterion and to investigate the determinant of the higher apparent molecular weight of the glycopeptides from these cells.

At the time of the assay, the diagnosis of patient 7 (Table 1) was uncertain. He was suspected of having leukaemia but since the Paul Bunnell test was positive, the possibility of IM could not be excluded. The elution profile of the surface glycopeptides indicated similarity with malignant disease. In the further course of the disease the patient developed the typical clinical picture of CML of the juvenile type.

This result prompted the investigation of lymphocytes from patients with IM. In all 10 cases studied (Table 2), the elution profiles of the glycopeptides coincided with controls of normal lymphocytes or exhibited a small shift to lower molecular weight regions (Fig. 4). Neuraminidase did not materially change the relative elution behaviour of the IM and normal glycopeptides.

The Pronase-digested, fucose-labelled glycopeptides obtained by mild trypsinisation of human leukaemic and lymphosarcoma cells consistently eluted ahead of the corresponding materials from resting or PHA-stimulated lymphocytes. The absence of such difference between resting and PHA-stimulated peripheral lymphocytes (Fig. 1) excludes the possibility that the change in tumour glycopeptides is due to blastoid transformation. The finding that glycopeptides from IM cells eluted together with or slightly after those from normal lymphocytes is further indication that the different behaviour of leukaemic cells is associated with their malignant character. Moreover, it excludes the possibility that the behaviour of the malignant lymphocytes is only the result of the presence of a high count of leukocytes in the peripheral blood since comparable or even higher counts were observed in IM patients. Also, the structural change in surface glycoprotein was consistently found in all malignant cell samples irrespective of their assumed origin as B cells (CLL Burkitt's lymphoma) or as T cells (childhood lymphosarcoma) whereas ALL (acute lymphocytic leukaemia), CML and AML cells lack B and T markers⁸.

Fig. 4 Elution profile of L-fucose-containing glycopeptides of Pronase-digested trypsinates, from differentially labelled peripheral lymphocytes from patient 3 (Table 2) with infectious mononucleosis (●) and from normal peripheral lymphocytes (○) following filtration through Biogel P10–Sephadex G-50 fine (2:1). The radioactivity of the fractions is represented as percentages of total radioactivity eluted from the column. The column markers blue dextran (BD) and phenol red eluted from the column in fraction range 28–33 and 86–94 respectively.



Thus we conclude that surface glycoprotein composition in human leukaemic and lymphosarcoma cells is characteristically changed relative to normal; that except for CML this change is due to an increased content of neuraminidase-sensitive sialic acid; and that the difference is observed in all transformed and malignant cells studied so far, irrespective of the cell type, natural history of the cell, species or oncogenic determinant. This glycoprotein change may be a universal property of tumour cells and related to the expression of the neoplastic phenotype. If not instrumental in neoplastic behaviour, it may at least have diagnostic value.

Finally, we want to point out that the experimental device used here monitors only a minor part of the sialoglycoprotein of the cell surface⁴. Therefore, our observations do not necessarily correlate with other changes observed in the total sialic acid content or its exposition on the cell surface, let alone that in these respects no universal changes have been observed in tumour cells^{9,10}.

We thank Mr J. Breckveldt for technical assistance and Miss Joke Blok and Mr H. van Rooy for their help in tissue culture experiments and Miss Loes Pullens, Mrs Mary Geel and Mrs Anneke van Dongen for collecting blood samples.

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Detoxifying effect of yellow substance on *E. coli* in media containing copper

DISSOLVED yellow organic matter (yellow substance) is ubiquitous in aquatic systems, and is thought to influence the state of ecosystems by affecting the transport and fate of minerals, primary production and, perhaps, the behaviour of aquatic organisms¹⁻⁴. Although much studied, yellow substance remains chemically and physically ill-defined. It is primarily responsible for the yellow colour of particulate-free natural waters which is generally accepted to be the result of the aqueous extract of decaying plant material. One of the primary constituents of plant material, lignin, is the precursor of a class of stable, yellow-brown, intermediate decay products called humins or humic substance⁵. Divided into two fractions, humic acid (base soluble) and fulvic acid (acid soluble), these compounds are insoluble in water and are typically analysed as extracts from soil or sediment. Yellow substance is the remaining yellow decay product and is often called soluble humin (dissolved humic acid). There are data, particularly differences in elemental

composition and visible and ultraviolet light absorption, however, suggesting that yellow substance is a separate compound with cellulose as its source rather than lignin⁶.

There have been two attempts to establish a direct biological role for yellow substance. Prakash and Rashid^{7,8} observed moderate enhancement of growth of various phytoplankton in cultures spiked with yellow substance (referred to as dissolved humic acid in this work); and, in similar experiments, Shapiro¹ reported possible growth enhancement in cultures of several algae. Other findings suggest an interaction with metals in the aquatic ecosystem⁷. Horne and Goldman⁸ reported the suppression of nitrogen fixation in blue-green algae by copper ions (Cu^{2+}). Jones⁹ postulated that metal ions, particularly Cu^{2+} in seawater media suppress *Escherichia coli* in that system. Erickson^{10,11} obtained similar results with *Thalassiosira pseudonana* with the added inference that decomposed natural plankton and detritus, possibly yielding natural chelators¹², reduced Cu^{2+} toxicity. We have now found that although yellow substance plays no direct role in population growth of *E. coli*, it does ameliorate the effects of Cu^{2+} added to the system in toxic concentrations.

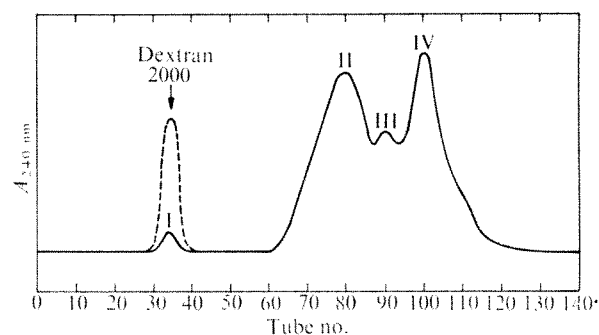


Fig. 1 Chromatogram of parent yellow substance used in this study. Flow rate was 11 ml h^{-1} . Fraction I, recovered at the exclusion limit, was colourless and was not included with parent yellow substance (II-IV).

Yellow substance was obtained from Millipore-filtered ($0.2 \mu\text{m}$) water from the upper Russian River in northern California in October 1973. Isolation was accomplished by ion exchange techniques designed to handle large volumes¹³. Further purification for removal of trace organic material such as free amino acids was accomplished by gel filtration on a 200-cm column of Sephadex G-50 with 0.02 M NaCl as eluant. Resulting chromatograms indicated the possibility of three major fractions (Fig. 1). Comparison of chromatograms of yellow substance obtained on Sephadex G-25 and G-50 with some known standards and with the exclusion limits of the gels indicates an approximate average molecular weight of 5,000. Amicon (UM-02) filtration and lyophilisation yielded an electrolyte-free product which represented approximately 0.001% of the mass of the original filtered water sample.

Cultures of *E. coli* W 1485 (ref. 14) were prepared in a medium containing, (g l^{-1}), K_2HPO_4 , 10.5; KH_2PO_4 , 4.5; MgSO_4 , 1.0; NH_4Cl , 1.0; and glucose, 2.0. The bacteria were cultured aerobically at 37°C with sterile conditions maintained throughout. Cell density was measured by absorbance at 650 nm with a Cary 16 spectrophotometer. For the experiments, all cultures were diluted to an initial optical density of 0.008. After an initial static period, cultures grew exponentially with a mean doubling time of $66.0 \pm 0.9 \text{ min}$ (average and standard deviation of 20 independent cultures). Growth was followed by absorbance measurement every 30 min out to an A_{650} reading of 0.4, representing approximately six generations of reproduction.

To measure the effects of yellow substance on the growth of the population we added it to the culture medium in con-

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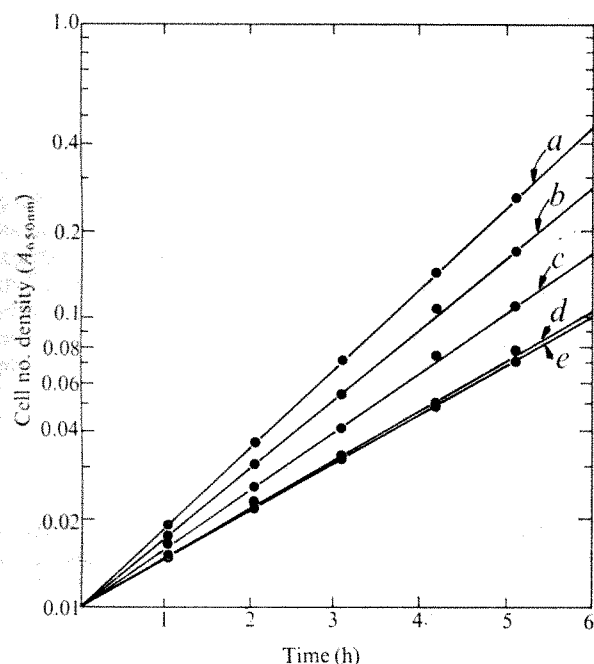


Fig. 2 *a*, Growth of *E. coli* in control media. *b-d*, Growth of *E. coli* in media made toxic with 6×10^{-6} M Cu^{2+} with additions of equimolar EDTA (*b*), yellow substance (*c*), citrate (*d*). *e*, Growth of *E. coli* in toxic control (6×10^{-6} M Cu^{2+}).

concentrations of 10^{-6} to 10^{-4} M, assuming an average molecular weight of 5,000 for the yellow substance. Doubling time was not affected by yellow substance. When glucose was replaced by yellow substance, growth stopped.

To investigate the influence of yellow substance on growth in media made toxic with cupric ions, we first established the growth suppressant effect of cupric ions on our *E. coli* system by measuring doubling times in cultures to which CuSO_4 (ultrapure, Johnson, Matthey and Co.) had been added at concentrations of 10^{-4} – 10^{-6} M. There was no growth in the presence of 10^{-4} M Cu^{2+} . At 6×10^{-6} M, growth remained linear but at a substantially reduced doubling time of 108 ± 2.2 min (Fig. 2).

The detoxifying effect of sodium citrate, EDTA and yellow substance was measured in the system made toxic with 6×10^{-6} M Cu^{2+} (Fig. 2). Concentrations of yellow substance of 10^{-5} M significantly enhanced growth rate beyond the toxic control (86 min doubling time compared with 108). Comparison of the doubling time with equimolar EDTA (76 min) and sodium citrate (108 min), indicate that yellow substance is considerably more effective in ameliorating Cu^{2+} toxicity than citrate and comparable with EDTA.

These results suggest that yellow substance should be considered as a prime moderator of metal ion availability to microorganisms in aquatic ecosystems. To ascertain the ultimate influence of yellow substance on ecosystems would require extensive investigation of the effects of the alteration of pH and redox potential on the results we have observed.

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A simple cytochemical technique for demonstration of DNA in cells infected with mycoplasmas and viruses

4'-6-DIAMIDINO-2-PHENYLINDOLE (DAPI), which was first synthesised by Dann *et al.*¹ as a trypanocide related to Berenil, has been shown to possess useful DNA binding properties². Thus, DAPI will bind differentially to yeast mitochondrial and nuclear DNA forming highly fluorescent complexes and enhancing the separation of the two DNAs in caesium chloride gradients². DAPI can also be used as a highly specific fluorescent stain for both nuclear and mitochondrial DNA in yeast. It seems to be highly sensitive and probably permits detection of a single yeast mitochondrial DNA molecule (D.H.W., and D. J. Fennell, unpublished).

We have investigated the use of DAPI in tissue culture systems and have found that it offers a very simple and sensitive procedure for detecting mycoplasma contamination.

Mycoplasmas are a major source of contamination of many cell lines carried in the laboratory, and there are many techniques available for detecting them (for review see ref. 3). Most of these methods are relatively lengthy and involve either cultivation of the mycoplasmas in special broth or agar, rather subjective assessments by cytochemical techniques, or some biochemical measurements often quite complex in nature. Probably the most sensitive and least ambiguous method presently available for the detection of mycoplasmas in tissue culture is that which uses the incorporation of ^3H -thymidine followed by autoradiography of the labelled cells⁴. Discrete and characteristic grains in the cytoplasm and on the cell surface are indicative of the presence of mycoplasmas. This method however, while sensitive and relatively straightforward, does not give the often necessary rapid diagnosis. We have thus developed a procedure for detecting mycoplasmas in tissue culture using DAPI which seems to have all the attributes of the autoradiographic method but is quicker and simpler.

On adding DAPI to tissue culture cells we found that it was rapidly taken up into cellular DNA yielding highly fluorescent nuclei and no detectable cytoplasmic fluorescence under the conditions used (Fig. 1a). If, however, the cells were contaminated with mycoplasmas, characteristic discrete fluorescent foci were readily detected in the cytoplasm and on the surfaces of the cells (Fig. 1b). Similarly, DAPI staining of cells infected with vaccinia virus revealed characteristic fluorescent virus 'factories' in the cytoplasm (Fig. 1c). The method has been applied to a variety of tissue culture cells (HeLa, KB, BHK21, MDBK, LLCMK2) and in all cases where mycoplasmas could be detected by the conventional technique of agar cultivation⁵, cytoplasmic fluorescence was readily observed. In some cases, moreover,

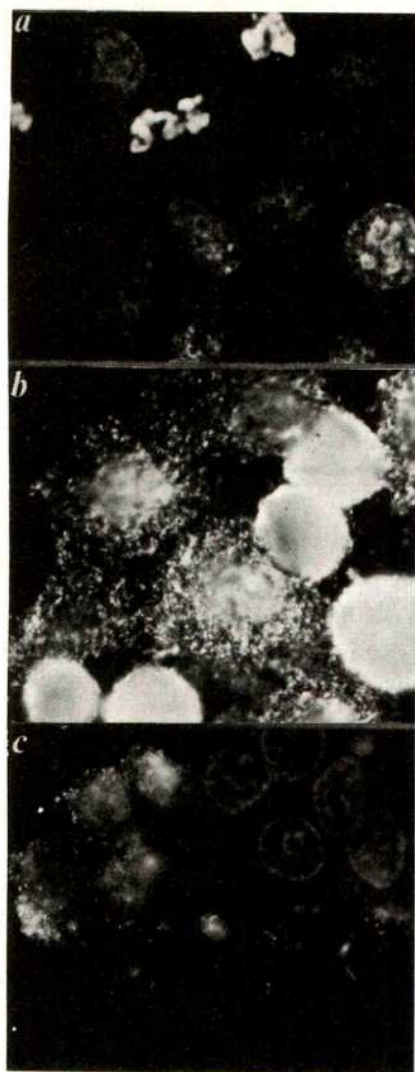


Fig. 1 *a*, Photomicrograph of control, unfixed and uncontaminated HeLa cells, treated with DAPI ($0.1 \mu\text{g ml}^{-1}$) at 37°C for 1 h. Fluorescent nuclei with some mitotic figures can be observed. *b*, Photomicrograph of unfixed, mycoplasma-contaminated HeLa cells treated with DAPI as in *a*. Characteristic cytoplasmic fluorescence can be seen; on refocusing on a different plane, the apparent intense nuclear fluorescence can be seen to be mainly cytoplasmic. *c*, Photomicrograph of unfixed HeLa cells 3 h after infection with vaccinia virus (at an added multiplicity of about 5 PFU per cell). Characteristic 'star-like' fluorescent clusters of presumably maturing virus in 'factories' can be observed ($\times 1,225$).

where there was no clear evidence by agar cultivation, cytoplasmic fluorescence could be observed in a minority of cells, probably indicative of a low level of mycoplasma contamination. A variety of conditions for the incorporation of DAPI into the tissue culture cells were examined, and while it seemed that a fairly wide spectrum of pH, ionic strength and concentration of DAPI could be used, the following simple procedure has been applied on a routine basis for monitoring cells for mycoplasma contamination.

Monolayers of cells grown on coverslips were washed once with phosphate buffered saline (PBS) and incubated in a small volume of PBS containing DAPI at a concentration of $0.1 \mu\text{g ml}^{-1}$ at 37°C for 15–30 min. The coverslip was again washed once with PBS and inverted (without fixation) on a microscope slide and examined in a fluorescence microscope (with a high pressure mercury vapour lamp as a source—for example, Vickers M41 Photoplan—with excitation frequency about 365 nm and emission principally about 450 nm). By lengthening the period of incubation in the presence of DAPI, the intensity of fluorescence in the

nuclei can be increased; with the shorter period of incubation there is less nuclear fluorescence and any cytoplasmic fluorescence is more easily monitored. Background fluorescence is minimal and thus there is no difficulty in discerning fluorescent DNA-DAPI complexes. That the fluorescent foci detected could be attributed to mycoplasmas was tested by deliberately infecting an uncontaminated line of HeLa cells. Mycoplasmas (*Mycoplasma fermentans* PG18) were grown in broth culture and titrated using the indicator techniques as previously described³. Monolayers of cells were infected at different added multiplicities and examined at various stages after infection and also after subcultivation of the cells. By using the DAPI technique it was possible both to follow the course of the mycoplasma infection of the cells and also to detect mycoplasmas in the broth cultures. The uninfected cells always gave negative cytoplasmic fluorescence during these procedures while the infected cells showed varying degrees and distribution of the characteristic cytoplasmic fluorescence.

This technique is extremely simple and can be readily applied to monitor tissue culture cells for mycoplasma contamination. Our observations also suggest that the technique may be more sensitive than conventional cultivation techniques and, of course, a cytochemical test obviates the requirements for fastidious culture media. The method should also be useful as a more general tool for monitoring the distribution and properties of DNA in a variety of biological contexts; for example, the course of vaccinia virus infection (Fig. 1c), as the virus matures in the cytoplasm, can be followed readily. We have also successfully used DAPI to follow the fate of inoculum adenovirus at early stages of infection.

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Note added in proof: Since submitting this letter, we have used as an alternative to DAPI, the fluorescent DNA-binding benzimidazole derivative Hoechst 33258 (ref. 6) with broadly similar results.

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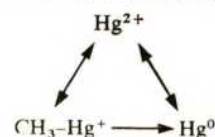
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Biosynthesis and degradation of methylmercury in human faeces

MERCURY is transformed by the microorganisms present in aquatic sediments¹ and rat intestines². Examples of some of the important reactions are illustrated below.



Divalent mercury is reduced to elemental mercury (Hg^0)

aerobically and alkylated to methylmercury (CH_3Hg^+) anaerobically. Methylmercury can be demethylated to Hg^0 and CH_4 aerobically or anaerobically. Methylmercury is of great concern to man because it is highly toxic, is absorbed through the intestinal wall 45 times more rapidly than Hg^{2+} (ref. 3) and is retained in the body longer than Hg^{2+} . The ingestion of food contaminated with methylmercury has resulted in severe outbreaks of mercury poisoning in Minamata, Japan, Alamogordo, New Mexico, and Iran. The ability of bacteria to alkylate mercury prompted us to investigate whether the normal microbial flora of the human intestine could synthesise methylmercury and thus contribute to the body burden of this noxious compound.

Samples of freshly voided human faeces were weighed and placed immediately in anaerobic culture tubes (Bellco glass). Previously reduced culture medium⁴ was added to make a 25% (w/v) suspension of the faecal matter. To avoid killing oxygen sensitive anaerobes, all manipulations were performed in an oxygen-free environment using procedures described by Hungate⁵. The faecal suspensions were gassed with a mixture of H_2 and CO_2 and isolated from air with rubber stoppers. The required amount of $^{203}\text{HgCl}_2$ (New England Nuclear Corp.) or $^{14}\text{CH}_3\text{HgCl}$ (NEN) was added to the gassed mixtures and rapidly mixed. The samples were incubated at 37°C in a shaking water bath. Methane synthesised by the faecal bacteria was measured by gas chromatography on a silicic acid column.

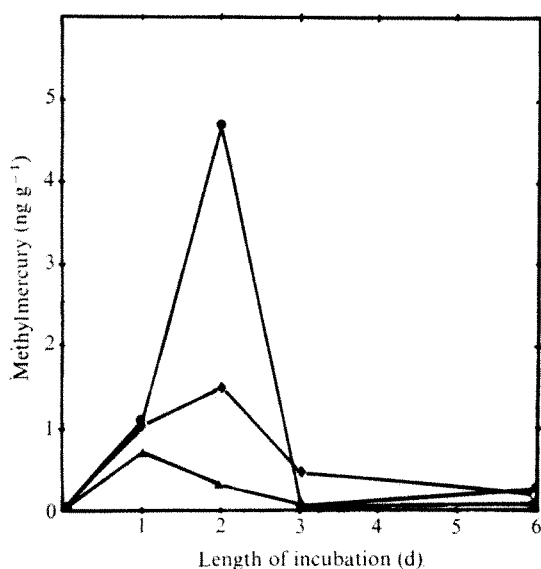


Fig. 1 Concentration of methylmercury in faeces after addition of 5×10^{-7} M HgCl_2 (Δ); 1×10^{-6} M HgCl_2 (\blacklozenge); and 7×10^{-6} M HgCl_2 (\bullet).

Methylmercury was extracted from the faecal suspension by the combined mercuric chloride–cysteine acetate method of Westöö⁶. One millilitre of the sample was placed in a centrifuge tube together with 1 ml HgCl_2 (5%, w/v); 1 ml 6N HCl, and 2 ml benzene. The sample was mixed vigorously for 1 min and then centrifuged at $3,000g$ for 5 min at room temperature. The upper (benzene) layer was removed and mixed vigorously with 0.5 ml of 1% cysteine acetate for 1 min. The aqueous layer was removed and mixed thoroughly with 0.2 ml of 6N HCl and 1.0 ml of benzene. The benzene layer was mixed with anhydrous sodium sulphate to remove water.

Methylmercury was identified by thin-layer chromatography of the cyanide and iodide salts. The cyanide salt was made by mixing the benzene layer from the cysteine acetate extraction with 0.5 ml of 1M KCN. The cyanide

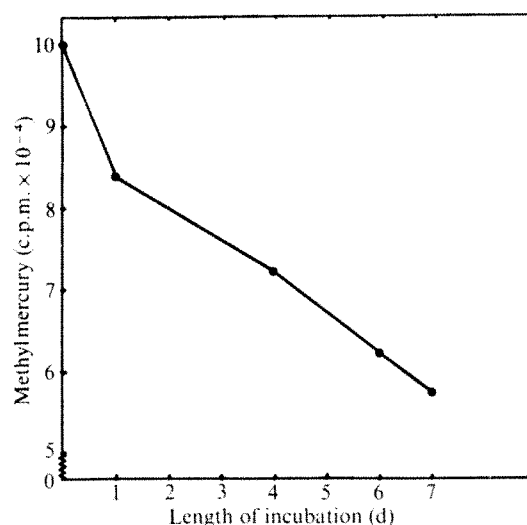


Fig. 2 Disappearance of $^{14}\text{CH}_3\text{Hg}^+$ from faeces. Initial methylmercury concentration was 3.1×10^{-6} M. The $^{14}\text{CH}_3\text{Hg}^+$ was added to anaerobic tubes containing 10 ml of diluted faeces. The tubes were gassed with $\text{H}_2 : \text{CO}_2$ (80 : 20) and incubated at 37°C . One ml samples were withdrawn and extracted for methylmercury as described.

derivative was then chromatographed on silica gel G by thin-layer chromatography using diethyl ether–toluene (90 : 10, v/v) as the developer. Methylmercury was visualised by spraying with 0.4% (w/v) dithizone in benzene. To verify further that the compound in question was methylmercury, the benzene layer was mixed with NaI and chromatographed using light petroleum–diethyl ether (70 : 30) as the developer. The presence of ^{14}C -methylmercury or ^{203}Hg -methylmercury (isotopic methylmercury) was established either by scanning the chromatographic sheets in an actigraph (Nuclear-Chicago) or by counting the amount of isotope in silica gel scraped from the sheets.

Methylmercury was produced when faecal matter was incubated anaerobically with $^{203}\text{HgCl}_2$ (Fig. 1). Maximum levels of methylmercury were produced during the first 2 d of incubation followed by a rapid decline. This type of production pattern was repeated in every sample examined, although the peak sometimes appeared as early as 18 h. The amount of methylmercury produced was directly proportional to the amount of Hg^{2+} added. In this experiment the amount of radioisotope was held constant and the amount of non-radioactive Hg^{2+} was varied. No dilution effect was observed, suggesting that a significant proportion of the Hg^{2+} was unavailable for methylation, probably because it was immobilised as the very insoluble sulphide derivative. It is interesting that there was no correlation between methane and methylmercury biosynthesis. Wood *et al.*⁷ reported that cell-free extracts of the methanogenic bacterium *Methanobacterium* strain M.o.H. could catalyse the alkylation of Hg^{2+} . Samples which did not synthesise methane, however, still yielded high levels of methylmercury.

The rapid loss of methylmercury from the samples prompted an investigation into the half-life of the compound in the faeces. These experiments were performed by following the disappearance of a known amount of added $^{14}\text{CH}_3\text{Hg}^+$. As Fig. 2 shows, $^{14}\text{CH}_3\text{Hg}^+$ disappeared at a constant rate during the 7-d test. Thus, either by chemical or biological means, methylmercury can be degraded in an anaerobic faecal specimen. $^{14}\text{CH}_4$ was not observed, thus loss is not the result of reductive demethylation.

These studies are evidence that the microbial flora of the intestine of man has the potential to transform Hg^{2+} to highly toxic methylmercury and could contribute significantly to the methylmercury burden of the body and thereby

add to the risk of incurring or increasing the severity of methylmercury poisoning.

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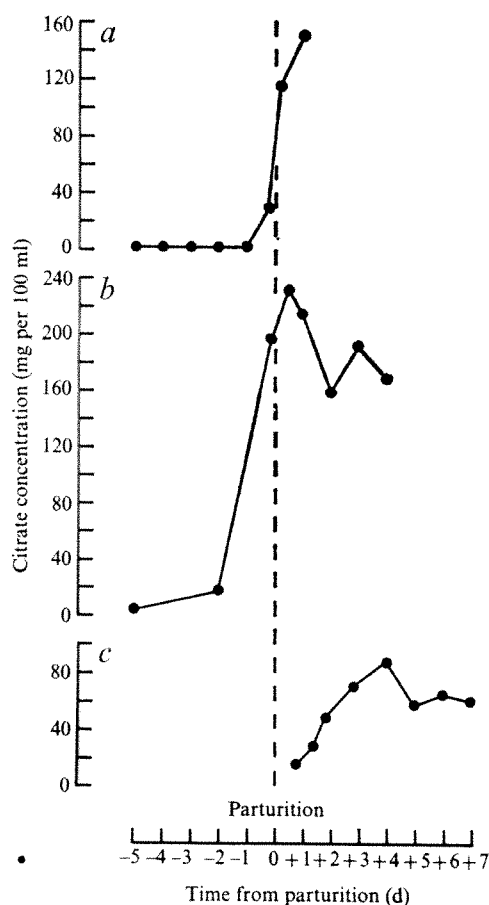
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Citrate in milk: a harbinger of lactogenesis

CONSIDERABLE amounts of citrate (130-160 mg per 100 ml) occur in the milk of cows and goats, and approximately half this amount in that of women. In spite of its central role in the metabolism of all cells, the significance of the presence of citrate in milk and its mode of secretion are not known¹. We report that the final stage of lactogenesis is preceded by the onset of citrate secretion into colostrum.

Matured but previously quiescent epithelial cells suddenly start to secrete large quantities of protein, fat and carbohydrate at about the time of parturition. Although there are significant changes in plasma hormone concentrations in late pregnancy and at term, the nature of this lactogenic trigger remains unknown^{1,2}. Cowie and Tindal³ have suggested that the presence

Fig. 1 Concentrations of citrate in the mammary secretion of *a*, a cow; *b*, a goat; and *c*, a woman about the time of parturition. Small samples were taken from the teat and analysed by the method of White and Davies¹⁰. In all species the young were suckled normally after parturition.



in the secretory tissue of the relevant organelles, enzymes, substrates and some specific milk components, is not sufficient evidence for the onset of copious secretion. For example, udders of cows and goats may contain several litres of a fluid with almost normal milk lactose and ion concentrations 6-7 weeks before term, although copious secretion starts at about the time of parturition (ref. 3 and I. R. Fleet, J. A. Goode, M. H. Hamon, M. S. Laurie, J. L. L., and M. P., unpublished). The onset of secretory activity therefore occurs in two stages, and other investigations^{4,5} have in fact only covered the first stage^{4,5}.

The rate of onset of copious secretion is indeed impressive; in cows the yield may be as high as 40 l d⁻¹ within a few days after parturition and 4 l d⁻¹ in goats. The mammary glands need about 70 g glucose per litre milk formed to support secretion⁶, and although mammary blood flow increases markedly before parturition in goats and cows^{7,8} the uptake of glucose and oxygen does not begin until immediately after parturition and then reaches high levels within a few hours⁸.

In milk from seven goats and seven cows, citrate concentration increased by 3-150 times (median for goats 10 times, for cows 46 times) 1-2 d pre-partum and 2-3 d post-partum (Fig. 1). This rapid increase is illustrated by data from a cow in which the citrate concentration increased from 7 to 110 mg per 100 ml in the last 5 h of pregnancy. In women the onset of copious milk secretion does not begin until 3-4 d post-partum⁹, and it is significant that citrate in samples of secretion obtained from the wife of a colleague on the day of delivery was low and then increased to reach a peak on day 4 (Fig. 1).

Milk citrate is synthesised from glucose and acetate in the mammary gland of the goat⁹. The sudden marked increase in citrate thus indicates not only the onset of synthesis and secretion of this substance but of copious secretion as well.

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Isoenzymes of hexokinase in human muscular dystrophy

OF the several genetically determined diseases called muscular dystrophy, the best known, most studied and most severe are the sex-linked Duchenne type and its more slowly progressing variant, the Becker type¹. The aetiology of these dystrophies is unknown, although dystrophies associated with deficiencies of vitamins and other nutrients are known in animals, and myopathy in man may arise from causes as diverse as alcoholism, thyrotoxicosis, infection and autoimmunity². Such variety of known myopathies gave little help to early searches for the biochemical cause of the genetically determined dystrophies³. Recent workers have turned to biochemistry in search of a specific lesion in protein synthesis and its control⁴⁻⁶, or to tissues other than muscle, for example, its motor innervation⁷⁻¹⁰ or vascular supply¹¹. Clearly, a genetic lesion must be translated

into some change in protein synthesis, but this change will have other effects which will probably be more readily detectable. Other tissues besides muscle will be subject to the genetic changes which determine dystrophy, and may also show pathological signs.

Our belief is that dystrophic skeletal muscle will have specific properties from which the primary lesion(s) may be deduced. We have found that in dystrophic muscle, more glucose than usual is converted to fructose instead of to glucose 6-phosphate, under certain metabolic conditions^{12,13}. The conversion of glucose to glucose 6-phosphate is catalysed by hexokinase (EC 2.7.1.1.) which is present in mammalian tissue in one or more of several electrophoretically distinguishable isoenzymes differing considerably in their respective K_m values for glucose¹⁴⁻¹⁶.

Hexokinase activity has previously been measured in extracts of normal and dystrophic human muscle¹⁷⁻¹⁹, and seems to be present in adequate amounts in the test tube at

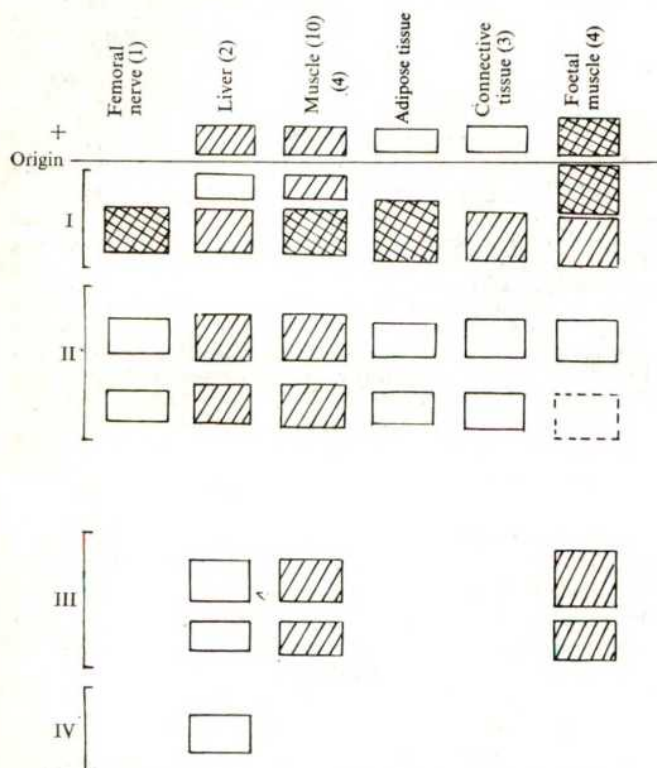


Fig. 1 Hexokinase isoenzymes of normal human tissues. Tissue samples (50–200 mg) were homogenised in 2–20 volumes of 0.1 M Tris, pH 7.4 containing EDTA (5 mM) and glucose (10^{-6} M). Homogenates were centrifuged for 6,000,000 g min. Supernatants were used directly for electrophoresis on Cellogel strips (Reeve Angel, London) at 200 V for 1 h in 0.02 M sodium barbital buffer, pH 8.4 containing EDTA (2.7 mM). All these procedures were carried out at 4°C. Staining strips for protein was carried out for 5 min in a solution of 0.2 g Ponceau S and 3 g trichloroacetic acid in 100 ml water. Staining of hexokinase activity took place at three different glucose concentrations for each extract, namely 10^{-2} M, 5×10^{-4} M and 10^{-6} M, according to the method of Katzen, Soderman and Wiley¹⁵. Supernatants were also concentrated tenfold using Minicon polymer blocks (Amicon Ltd, High Wycombe, UK) and again subjected to electrophoresis so that faintly-staining bands should not be overlooked. Bands of hexokinase activity were referred for comparison to the albumin band of the strip stained for protein. The degree of activity is represented by the density of shading. Tissue was used either fresh or after storage at -70°C . Liver and nerve were autopsy specimens. Muscle (four spinalis; six latissimus dorsi) was obtained during orthopaedic operations, and connective tissue and adipose tissue used in this study were trimmed from around such muscle specimens. Foetal muscle was obtained from embryos at therapeutic abortion at 14–18 weeks gestation. Isoenzymes are classified according to Katzen *et al.*¹⁵. The number of specimens used is given in parentheses. There was no detectable difference in the isoenzyme patterns of spinalis and latissimus dorsi muscles.

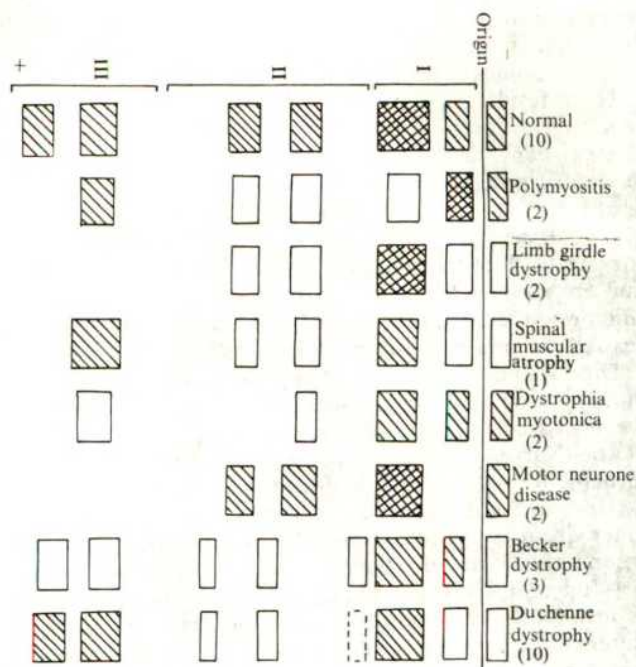


Fig. 2 Hexokinase isoenzymes in human muscle in health and disease. Specimens (10–100 mg) from diagnostic muscle biopsies were all obtained from deltoid, except for four patients with Duchenne dystrophy and two with Becker dystrophy in which the site of biopsy was gastrocnemius. Deltoid muscle was obtained at autopsy from three patients dying at age 18–20 yr from the effects of Duchenne dystrophy. These muscles were grossly affected, and muscle fibres were dissected from the surrounding fibrous and fatty tissue under magnification. The remaining patients with Duchenne dystrophy aged 6–10 yr were still ambulant; microscopic examination of the muscle showed active myopathy. The patients with Becker dystrophy were aged 8–18 yr, all actively ambulant, and showed early myopathic histopathology. Other patients used as controls were of widely differing ages. Treatment of specimens was otherwise as for Fig. 1.

the high glucose concentrations used. We examined the isoenzyme patterns of hexokinase in a variety of tissue samples. Figure 1 shows the normal isoenzymes of human tissues, illustrating the variation in isoenzyme constitution between tissues but the constant electrophoretic mobilities of the bands concerned. Figure 2 demonstrates that the isoenzymes of diseased muscle, while varying in amount, do not differ from the normal isoenzymes in mobility, except in the case of the X-linked dystrophies of Duchenne and Becker. Normally, isoenzyme II consists of two bands on Cellogel, but in X-linked dystrophy there are three, two of which move faster than the bands of normal isoenzyme II, and one more slowly moving band close to isoenzyme I. Isoenzyme II is invariably weakly staining in these X-linked dystrophies, and is sometimes seen only after concentrating the extract. These differences indicate that the protein reacting as isoenzyme II in X-linked dystrophy differs from the normal isoenzyme II. As this isoenzyme is characteristically abundant in skeletal muscle, the change in its properties in dystrophy may be very significant. The alteration in isoenzyme II is not the consequence of adulteration with adipose or connective tissue, nor of reversion to foetal protein type, as these tissues contain only normal isoenzyme II. Furthermore, the change in isoenzyme II has also been shown in three livers, one brain and one sciatic nerve obtained *post mortem* from patients affected with Duchenne dystrophy, and from muscle and liver from a foetus of 18 weeks presumed to have Duchenne dystrophy.

In mixed extracts of normal and dystrophic muscle, the electrophoretic patterns of each constituent tissue were preserved. It was not possible to resolve the normal iso-

- enzyme II bands from the slowest and medium bands of dystrophic muscle isoenzyme II but the fastest band of dystrophic muscle isoenzyme II was clearly resolved.

These results suggest that the phenomenon expresses the genetic constitution of the tissues and is not the result of changes consequent upon disease, although it might be a secondary reflection of a yet more general genetic manifestation. Taken in conjunction with our other results^{12,13} it seems more likely that this alteration in hexokinase isoenzyme II, if confirmed, may imply a direct genetic alteration in its catalytic properties, possibly in its substrate affinities, which might restrict glucose flow through the hexokinase step in tissues where isoenzyme II is required.

This would then account for the observed fructose shunt^{12,13} and suggests an aetiology which could embrace the neurogenic⁷, myogenic²⁰ and even the vascular⁸ hypotheses. Nerve damage would be expected to occur from sorbitol formation, as described by Ward, Baker and Davies²¹ in diabetes; muscle would, as suggested earlier^{12,13} suffer from a gradual deposition of triglyceride in and around the fibres; deleterious effects on arterial or capillary function might be envisaged. The involvement of liver as mentioned by Thomson²² would be expected, while the pattern of muscular weakness and degeneration as described macroscopically by Bonsett²³ and microscopically by others^{24,25} would follow if the pathological changes were related to the effects of metabolism of glucose by muscle rather than alternative substrates, such metabolism being consequent on muscular activity²⁶.

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Possible role of prostaglandin F_{2α} in mediating effect of prolactin on RNA synthesis in mammary gland explants of mice

Cyclic nucleotides seem to be intimately involved in the initiation of lactation and the consequent production of milk substances in the mammary gland¹⁻⁵. The ratio of cyclic GMP

to cyclic AMP increased several-fold in mammary glands taken from rats immediately after parturition and during lactation^{1,2}. Further, dibutyl cyclic AMP attenuated or abolished several hormone effects on enzyme activities and other metabolic events in explants from mid-pregnant rats³. It seems that the regulation of the metabolism of the mammary glands of mice may be specifically related to the mechanism whereby prolactin affects this tissue^{4,5}: cyclic GMP mimicked the early action of prolactin on RNA synthesis in mammary gland explants, whereas dibutyl cyclic AMP and agents which inhibit phosphodiesterase abolished this effect. These observations suggest that the early effect of prolactin on RNA synthesis is mediated by an elevated level of cyclic GMP and a reduced or maintained level of cyclic AMP.

Table 1 Effect of various combinations of prolactin, PGF_{2α}, PGE₁, PGA₂ and indomethacin on RNA synthesis in mouse mammary gland explants

Agent added	³ H-uridine incorporation into RNA (d.p.m. per µg RNA)			
	Control	Prolactin	Agent	Agent + prolactin
PGF _{2α}	216 ± 18	395 ± 27	354 ± 18	353 ± 18
PGE ₁	193 ± 16	285 ± 22	182 ± 10	181 ± 19
PGA ₂	198 ± 23	392 ± 28	173 ± 21	171 ± 21
Indomethacin	275 ± 24	462 ± 36	280 ± 32	332 ± 36

Mammary gland explants from mid-pregnant Swiss-Webster mice were preincubated for 2 d in medium 199 containing insulin (2.5 µg ml⁻¹) and hydrocortisone (2.5 µg ml⁻¹) by methods described previously⁷. A PG (50 µg ml⁻¹), indomethacin (10 µg ml⁻¹) and/or prolactin (2.5 µg ml⁻¹) were then added to the explants and incubation was continued for 4 h. ³H-uridine (1 µCi ml⁻¹, 5 Ci mmol⁻¹) was added to the flasks 30 min before terminating the incubations. The specific activity of ³H in the tissue RNA was determined as before⁷.

Numbers are means ± s.e. of explants from seven flasks.

Several investigators have postulated⁶ that the actions of the prostaglandins (PGs) in several biological systems are mediated by cyclic nucleotides. Specifically, it has been postulated that the actions of the PGs of the F series are mediated by cyclic GMP while those of the E and A series are mediated by cyclic AMP. I have therefore tested whether the PGs of the A, E and F series would mimic or attenuate the effect of prolactin on RNA synthesis in mouse mammary gland explants. Table 1 shows that PGF_{2α} mimicked the action of prolactin on RNA synthesis while PGE and PGA₂ abolished the prolactin effect. Moreover, since maximally stimulatory concentrations of prolactin and PGF_{2α} were used, the non-additivity of the two effects is compatible with the possible mediatory role of PGF_{2α} for prolactin. The abolition of the prolactin effect by PGE₁ and PGA₂ may be the result of stimulation of adenylyl cyclase⁶ with a consequent enhanced rate of synthesis of cyclic AMP.

Since indomethacin is known to inhibit prostaglandin synthesis through inhibition of the prostaglandin synthetase enzyme, I tested the effect of this drug on the prolactin stimulation of RNA synthesis. Table 1 shows that indomethacin abolished the prolactin effect. These data, therefore, further support the hypothesis that the effect of prolactin on RNA synthesis is mediated by PGF_{2α}. Although an effect of indomethacin unrelated to its effect on prostaglandin synthesis may

Table 2 Time-course for the PGF_{2α} stimulation of RNA synthesis in mouse mammary gland explants.

Incubation time (h)	³ H-uridine incorporation into RNA (d.p.m. per µg RNA)	
	Control	PGF _{2α}
2	280 ± 22	283 ± 13
4	281 ± 16	365 ± 19

Incubation conditions were the same as those described for Table 1 except that the final incubation time was varied. Numbers are means ± s.e. of explants from 14 flasks.

explain the abolition of the prolactin effect, this seems unlikely since indomethacin did not attenuate the effect of $\text{PGF}_{2\alpha}$ on RNA synthesis (data not shown).

Further studies (Table 2) showed that the time-course for the effect of $\text{PGF}_{2\alpha}$ on RNA synthesis did not differ from that reported for prolactin⁷. The effect of $\text{PGF}_{2\alpha}$ on RNA synthesis was apparent after a 4-h incubation but not after a 2-h incubation.

These observations therefore suggest that $\text{PGF}_{2\alpha}$ may be the 'first messenger' for the action of prolactin on RNA synthesis in the mammary gland, whereas cyclic GMP may function as the 'second messenger'. It is interesting that lactation can be initiated in rats⁸ and humans⁹ when parturition is induced by administration of $\text{PGF}_{2\alpha}$. It thus seems likely that after parturition under physiological circumstances, prolactin may contribute to the initiation of lactation by a stimulation of the production of $\text{PGF}_{2\alpha}$.

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Endogenous protein kinase activity in nuclear RNP particles from HeLa cells

To elucidate the mechanisms which achieve and regulate the transport from the nucleus to the cytoplasm of those sequences of heterogeneous nuclear RNA (HnRNA) destined to become cytoplasmic mRNA, we have started a systematic investigation of the proteins associated with these RNA species in the form of the so-called 'RNP particles'. The general occurrence of such ribonucleoprotein complexes in eukaryotic cells is widely recognised although they have only recently been described in HeLa cells^{1,2}. The occurrence of phosphorylated proteins in rat brain nuclear particles³, together with the observation that mRNA release from adenovirus-infected KB cell nuclei *in vitro* is an ATP-dependent process⁴, has prompted a search for protein kinase activity in HeLa nuclear RNP particles. The role of protein phosphorylation in the regulation of enzyme activity is now recognised⁵ and we have observed such an activity which does not require the addition of exogenous substrates like histones. The endogenous protein substrates present in HeLa nuclear RNP particles have also been characterised.

RNP particles were spontaneously released from HeLa nuclei during incubation at 37°C (refs 1 and 6) and purified through a sucrose cushion. After incubation in the absence of added protein substrate, these particles are able to catalyse incorporation of radioactive phosphate from $\gamma\text{-}^{32}\text{P}\text{-ATP}$ into acid-insoluble form, the extent of incorporation being proportional to the amount of particles expressed as protein (Fig. 1a). This material was unambiguously identified as phosphoprotein by its insolubility in hot trichloroacetic acid, sensitivity to Pronase and resistance to ribonuclease. Cyclic AMP had no detectable

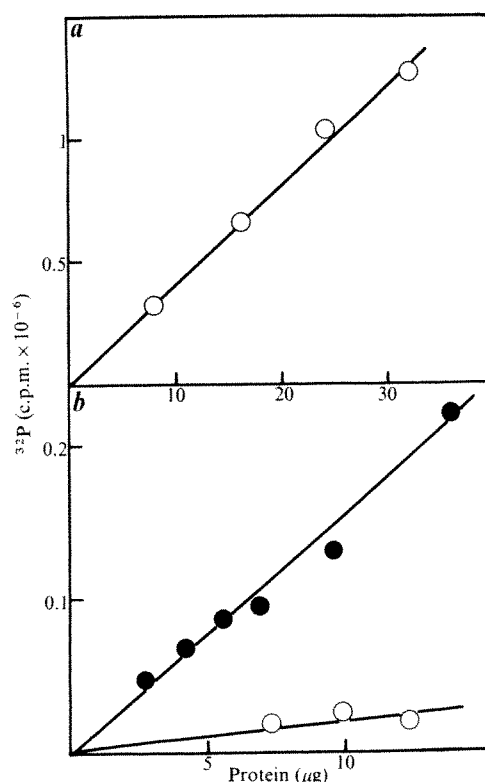


Fig. 1 Protein kinase activity in HeLa nuclear RNP particles. HeLa nuclear particles were prepared from 10^6 cells essentially as described¹. These 'native particles' were divided into two fractions. One half was kept at 0°C in 0.2 ml TM buffer (0.1 M Tris-HCl (pH 7.8), 10 mM MgCl_2) until use, whereas the other was incubated overnight in TM containing 0.5 M KCl at 0°C. This latter fraction was poured over a 3 ml sucrose cushion (20% w/v) in the same buffer and centrifuged at 55,000 r.p.m. for 4 h at 4°C in the SB405 rotor of an IEC B 60 ultracentrifuge. The pellet of 'washed particles' was resuspended in 0.2 ml TM buffer. The supernatant above the sucrose cushion (0.3 ml) was referred to as 'particles wash'. Protein determination was performed according to Lowry *et al.*⁷ using bovine serum albumin as standard. Reaction mixtures (0.1 ml) containing 2 nmol $\gamma\text{-}^{32}\text{P}\text{-ATP}$ (ref. 8) in TM buffer were incubated at 37°C for 15 min.⁹ The reaction was stopped with 10% trichloroacetic acid, the precipitates collected on glass filters and counted in a Tricarb scintillation spectrometer. *a*, Kinase activity in 'native particles'; *b*, kinase activity in 'washed particles' (○) and 'particles wash' (●).

effect on the rate or the extent of the phosphorylation reaction when assayed in the absence of theophyllin. Among the heterogeneous pattern of proteins associated with HnRNA in HeLa nuclei^{1,2}, a major species of molecular weight about 40,000 can be preferentially, although not exclusively, detached from RNP particles by 0.5 M NaCl³. Kinase activity was found to be localised exclusively in the 0.5 M KCl 'particles wash' (Fig. 1b) but not 'washed particles'. As in the case of complete particles, the reaction did not require exogenous substrate, such as histones, indicating that the 'particles wash' contains both the enzyme and its substrate. The absence of detectable activity in washed particles, however, could also reflect the complete removal of the endogenous substrate in addition to that of the enzyme itself. The fact that addition of H1 histone from calf thymus to washed particles did not significantly increase the amount of ^{32}P incorporated (not shown) renders the first possibility very unlikely unless the enzyme has such a restricted specificity as to be unable to phosphorylate any protein but its own cognate substrates. Although no kinase activity could be detected in the 'washed particles', it is apparent from the comparison of ordinates in Fig. 1a and b that the specific activity of the wash fraction is lower than that of untreated particles. This may result from either a greater enzyme stability and/or a more favourable spatial relationship with the substrates in the 'native particles' than in the soluble 'particles wash'.

We therefore characterised, using SDS-gel electrophoresis, the phosphoproteins produced *in vitro* by native particles (Fig. 2b), and by the 0.5 M KCl wash (Fig. 2d), with reference to the corresponding densitograms of stained gels (Fig. 2a and c). The protein pattern of native nuclear particles (Fig. 2a) is similar to those previously reported^{1,2}, whereas that of the 'particles wash' (Fig. 2c) shows a considerable enrichment in protein species of about 40,000 daltons in agreement with Pederson's report that 'washed particles' are preferentially depleted in this protein species². The distribution of ³²P radioactivity shows two discrete peaks corresponding to molecular weights of about 85,000 and 40,000 for both native particles and 'particles wash'. It has consistently been observed that the 85,000-dalton species is preferentially phosphorylated in the wash fraction while the reverse is true in 'native particles'. A closer association of the kinase with the 40,000-dalton species

in 'native particles' can be invoked. An additional phosphorylated species of molecular weight 26,000 has occasionally been observed and this may reflect the occasional presence in some particle preparations of an adventitiously bound protein, possibly a histone, from another nuclear compartment. This point is currently being investigated.

We have described the occurrence of protein kinase activity in the nuclear RNP particles from HeLa cells which can be selectively detached, together with its endogenous substrates, by 0.5 M KCl. A few discrete protein species seem to become phosphorylated in the reaction. While this work was being completed, a similar endogenous protein kinase activity has been reported in rat liver nuclear particles¹² which is able to phosphorylate polypeptides with molecular weights of 25,000, 36,000 and 42,000.

The main proteins released by washing the particles with 0.5 M NaCl have molecular weights of about 40,000 (Fig. 2c and ref. 2). We have recently isolated, using an affinity chromatography on poly(A)-Sephacryl column, a phosphorylated protein from HeLa cytoplasm whose size in SDS-acrylamide gels is close to the above value¹⁰. Preliminary experiments indicate that this protein fraction contains kinase activity (unpublished results).

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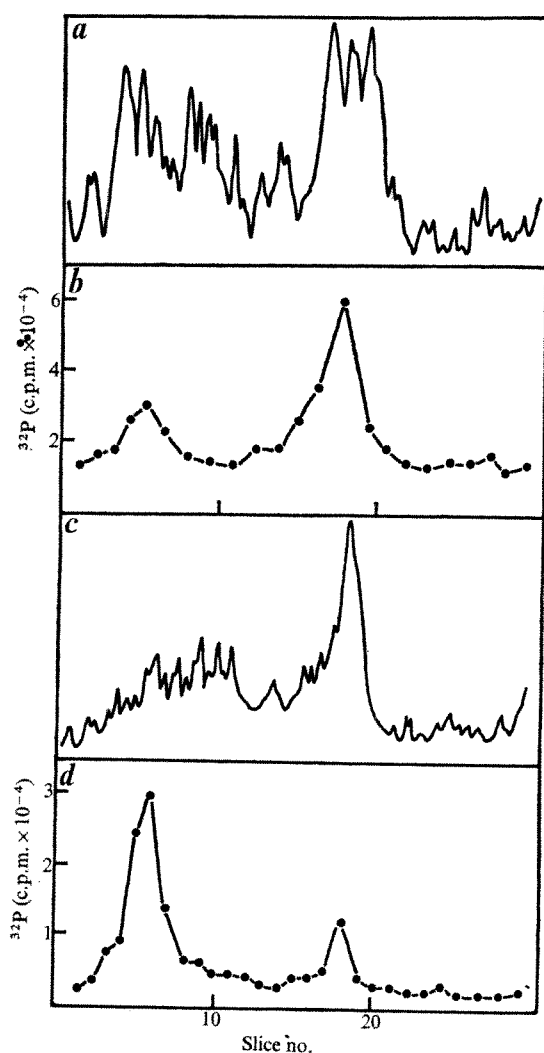


Fig. 2 Sodium dodecyl sulphate-acrylamide gel electrophoresis of proteins from 'native particles' and 'particles wash' incubated *in vitro* with γ -³²P-ATP. Protein from 'native particles' (80 μ g) and 'particles wash' (60 μ g) were incubated in 100 μ l TM buffer in the presence of γ -³²P-ATP as described in the legend to Fig. 1. Denaturation of the samples, electrophoresis on 10% acrylamide gels containing 0.1% sodium dodecyl sulphate, staining of the gels and densitometer tracing were carried out as previously described.¹⁰ Gels were then cut into 1 mm slices and counted directly in a Packard-Tricarb scintillation spectrometer using Cerenkov radiation. Molecular weights were estimated according to Weber and Osborn¹¹, using bovine serum albumin, aldolase and human γ globulin as standards. Densitometer tracings of proteins from 'native particles' (a) and 'particles wash' (c). ³²P radioactivity incorporated into proteins from 'native particles' (b) and 'particles wash' (d).

Analysis of X-ray-induced chromosomal translocations in human and marmoset spermatogonial stem cells

ONE of the major classes of genetic damage produced by ionising radiations is heritable reciprocal translocation. In a series of earlier publications, Brewen and Preston¹⁻³ demonstrated that each of several mammalian species has its own unique sensitivity to translocation induction in peripheral leukocytes and that human and marmoset leukocytes, although approximately equal in sensitivity, were twice as sensitive as mouse leukocytes. Brewen and Preston⁴ also reported that in the Chinese hamster and the mouse, the frequency of the translocation type analysed in the peripheral leukocytes was three to four times as high as the frequency of heritable translocations produced in spermatogonial stem cells and recovered in primary spermatocytes. As the mouse is the mammal currently used as the model for estimating man's genetic risk from mutagenic exposure, Brewen and Preston's earlier observations on germ cells should be extended to include a primate and man if possible. Here we present an extension of the interspecific and somatic against germ cell comparisons.

Mature male marmosets were testicularly irradiated with acute doses of 25, 50, 100, 200, or 300 rad of 250 kV X rays. At various intervals after irradiation, depending on the X-ray dose,

bilateral castration was performed, and preparations were made by the method of Evans *et al.*⁵, as modified by Chandley (personal communication). Biopsy material was also obtained from nine men, six of whom had received testicular X-ray exposures of 78, 200, or 600 rad. Details of the irradiation regime are given by Heller and Rowley⁶. The interval between exposure and sampling varied among the individuals, because the programme had been in progress for some time before the cytological investigation was started. Preparations were made by the same procedure as that used for the marmosets. Human and marmoset diplotene-diakinesis figures were analysed for the presence of multivalent configurations (Fig. 1) which were the result of reciprocal translocations induced in spermatogonial stem cells.

The results of this meiotic analysis are presented in Table 1. Previously published data⁷ from mouse studies are also presented in Table 1 for the sake of comparison. Yields of dicentric chromosomes, calculated from the coefficients of regression curves¹, in peripheral leukocytes at the same doses are presented for each species in order that somatic against germ cell comparisons can be made.

The data indicate that, as in the case of the peripheral leukocyte studies, both man and marmoset are at least twice as sensitive to reciprocal translocation induction in spermatogonial stem cells as the mouse. The ratio of somatic cell translocations (that is, dicentrics) to germ cell translocations is approximately 2:1 in man and marmoset at X-ray doses of 100 rad and lower. This variation from the mouse data might be explained by the greater efficacy in observing reciprocal translocations in the primates because the increased chiasmata frequency in primates

Fig. 1 Examples of diplotene-diakinesis figure in marmoset and human primary spermatocytes. *a*, Normal marmoset; *b*, ring-of-IV marmoset; *c*, ring-of-VI marmoset; *d*, normal human; *e*, ring-of-IV human; *f*, ring-of-VI human.

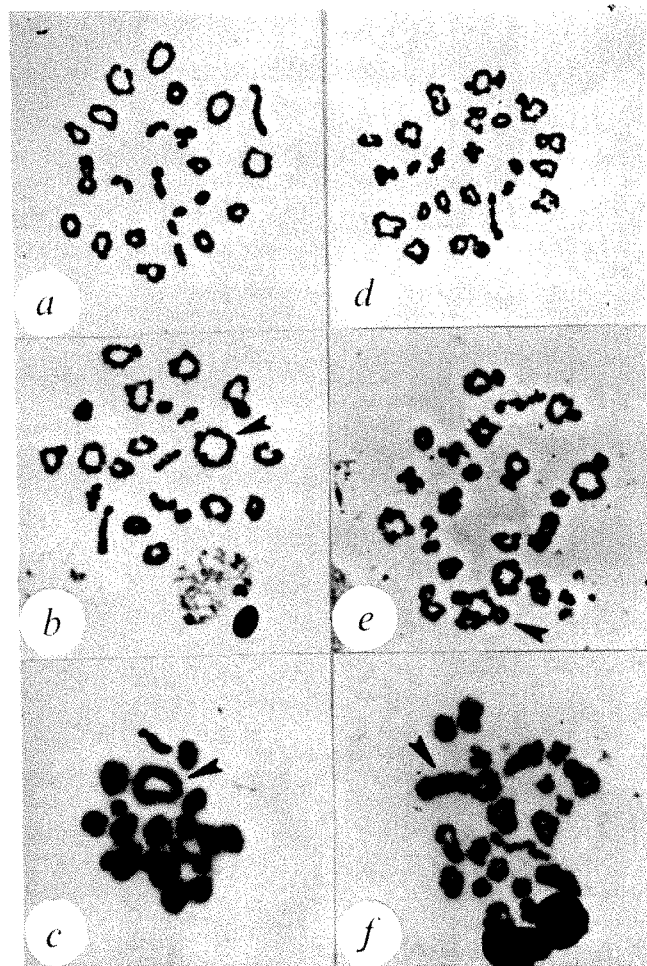


Table 1 Frequencies of reciprocal translocations in meiotic cells and dicentrics in peripheral leukocytes following X-ray exposure to man, marmoset, and mouse

Dose (rad)	Man		Marmoset		Mouse	
	% Dicentrics	% Reciprocal translocations \pm s.e.*	% Dicentrics	% Reciprocal translocations \pm s.e.†	% Dicentrics	% Reciprocal translocations \pm s.e.‡
0	0.0	0.0	0.0	0.0	0.0	0.0
25	—	—	3.0	2.8 ± 0.7	0.9	—
50	—	—	6.7	3.3 ± 0.8	2.1	0.7 ± 0.3
78	8.0	4.0 ± 1.0	—	—	—	—
100	—	—	16.3	7.8 ± 1.1	5.5	1.5 ± 0.5
200	35.2	7.0 ± 1.3	44.6	7.5 ± 1.9	16.4	4.3 ± 0.8
300	—	—	84.9	7.0 ± 1.1	32.7	7.8 ± 1.1
600	250.0	6.1 ± 1.8	—	—	114.0	19.5 ± 1.6

* The number of cells analysed at 78, 200, and 600 rad were 371, 300, and 180, respectively.

† A total of 600 cells were analysed from four testes at each dose, except for 200 rad where 200 from two testes were analysed.

‡ 600 cells were analysed at each dose.

will give a higher probability of a multivalent association being maintained at diplotene-diakinesis. The dose-response characteristics of translocation induction in the germ cells of man and marmoset are similar to those of the mouse. For all three, a peak yield is reached with an apparent decrease at higher doses. This peak yield, however, occurs at approximately 100 rad in the primates whereas it occurs at 500–600 rad in the mouse.

Using the translocation frequencies observed at doses of 100 rad and lower, the mean rate of reciprocal translocation induction in man and marmoset is 7.7×10^{-4} translocations per cell per rad. Using the data of Ford *et al.*¹⁸ on the rate of transmission of cytologically scored translocations to F_1 offspring of irradiated male mice as the lower limit of transmission (that is 1:8), and the expected frequency of transmission of 1:4 as the upper limit, a calculation of transmissible reciprocal translocations per gamete per rad can be made. This value is 0.96×10^{-4} – 1.93×10^{-4} translocations per gamete per rad for acute X-ray exposures.

Jacobs⁹ and Hamerton¹⁰ have summarised the data on the frequency of translocations in live births. The figures are: 0.06% reciprocal translocations, 0.08% Robertsonian translocation, and 0.03% unbalanced translocations. Jacobs *et al.*¹¹ have estimated that the spontaneous rate of viable translocations per gamete per generation is about 4×10^{-4} , of which 2.8×10^{-4} are balanced and 1.2×10^{-4} are unbalanced. This estimate is a minimum as not all translocations can be detected by somatic cell chromosome analysis. So, if it is assumed that the measured spontaneous rate is between 0.25 and 1.00 of the true spontaneous rate, the estimated doubling dose for the induction of reciprocal translocations by acute X rays is 2–16 rad.

In estimating a doubling dose for protracted (that is, chronic) exposures, one precaution must be taken. The experiments to date, using low dose rate irradiation, have been done with large total doses where the dose rate effect is maximised. In making risk estimates the principle concern is for low dose levels where, according to theory and experimental evidence, the dose rate effect for low LET radiations is minimal because of the small contribution by the two-track component. Thus a conservative estimate of a doubling dose for low-dose chronic X-ray exposure might be expected to be the same as for acute low doses with a maximum of 4–30 rad.

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Estimation of accessibility of DNA in chromatin from fluorescence measurements of electronic excitation energy transfer

EUKARYOTIC DNA is complexed with proteins which influence its conformation, stability and function^{1,2}. Attempts to correlate the chemical and biological properties of chromatin have focused on the coverage of DNA by chromosomal proteins³⁻⁸. As much as 50% of the DNA in chromatin is accessible to precipitants (such as basic dyes or polylysine)³⁻⁶ or to limiting nucleolytic digestion^{3,4,6-8}, suggesting a model of segmental DNA accessibility in chromatin resembling a frayed telephone wire. Digestion extensively alters chromatin structure, however, and the results of experiments involving precipitation or digestion may be influenced by processes associated with the aggregation phenomena serving as experimental endpoints. We have used an alternative approach to characterise DNA accessibility in chromatin using the transfer of electronic excitation energy between pairs of fluorescent dyes bound to chromatin at low ratios of dye to phosphate. The results suggest that at least one-third of the DNA in chromatin retains a high affinity for basic dyes. The exclusion of dyes or other molecules⁹ from portions of chromatin may, however, depend on the conformation of chromatin as well as on the protein-DNA stoichiometry.

The dyes quinacrine, acridine orange and 33258 Hoechst were chosen as energy donors. All three bind tightly to DNA and chromatin, exhibiting a fluorescence emission spectrum that extensively overlaps the absorption spectrum of the energy acceptor used, ethidium bromide. Following excitation of the donor, energy will be transferred with a high probability to ethidium molecules bound within a critical distance (approximately 25-35 Å) of the donor. Energy transfer efficiency decreases sharply with donor-acceptor separations above this value. The critical donor-acceptor distance for 50% efficient energy transfer, R_0 , determined by individual spectroscopic parameters and relative dye orientations^{10,11}, differs little between DNA and chromatin complexes with any particular donor-acceptor pair, although it varies somewhat between the three donor-acceptor pairs. The relative efficiency of energy transfer between fixed amounts of donor and acceptor bound to either DNA or chromatin depends on the probability that an acceptor molecule will bind within R_0 of the donor and thus, to a first approximation, this energy transfer varies inversely with the acceptor binding space. Hence it provides an estimate of DNA accessibility in chromatin.

Energy transfer in any particular region of chromatin (of length $\gg R_0$) should be proportional to the product of the binding affinities of the donor and acceptor to this segment and will thus reflect the correlation between donor and acceptor binding. Results averaged over many regions will also be weighted according to variations in energy donor fluorescence efficiency. For an accurate estimate of the acceptor binding space, the acceptor should bind to all regions accessible to the donor and the acceptor should not bind preferentially to particular regions of DNA. Quinacrine and ethidium bromide

seem to constitute a suitable donor-acceptor pair, since they both bind to DNA by intercalation in a mutually competitive manner with little base-composition specificity¹²⁻¹⁵.

If ethidium bromide is added to a quinacrine-DNA complex, quinacrine fluorescence is quenched by energy transfer to nearby ethidium molecules, which concomitantly exhibit sensitised fluorescence. At low donor and acceptor saturations, under conditions such that virtually all dye molecules are bound, the donor fluorescence intensity (F) varies inversely with the amount of added acceptor (Fig. 1). The amount of ethidium bromide necessary for a 50% reduction in donor fluorescence (ethidium bromide)₅₀ increases in proportion to the total accessible DNA phosphate concentration (Fig. 2). Quenching of the fluorescence of quinacrine bound to chromatin occurs at about one-third of the amount of added ethidium bromide needed for a comparable effect on quinacrine-DNA complexes (Fig. 2). Direct measurements of quinacrine binding¹⁵ indicate that calf thymus chromatin also possesses about one-third the number of quinacrine binding sites found in an equal amount of DNA, consistent with the existence of an extensive overlap between quinacrine and ethidium bromide binding spaces in chromatin.

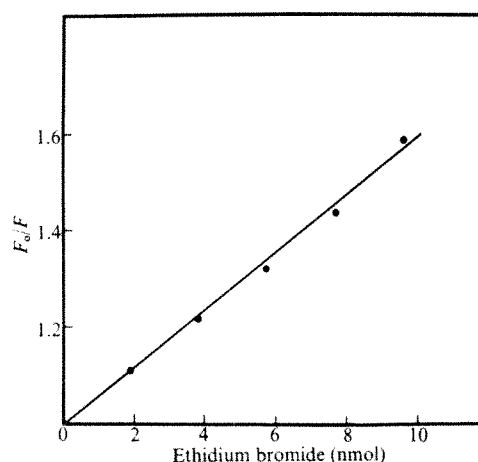


Fig. 1 Energy-transfer quenching of quinacrine fluorescence by ethidium in complexes with calf thymus DNA. The fluorescence of a 2-ml sample of a 0.7×10^{-6} M solution of quinacrine complexed with 2×10^{-4} M calf thymus DNA (in 0.01 M NaCl, 0.005M HEPES, pH 7) was measured after successive 20- μ l additions of a 1×10^{-4} solution of ethidium bromide. Excitation was at 420 nm. Quinacrine fluorescence (F), measured at 493 nm, was corrected for dilution by the ethidium bromide solution and for a very small amount of quenching due to the trivial process of fluorescence and reabsorption. F_0 is the initial fluorescence in the absence of added ethidium. The ratio of F_0/F is plotted against the amount of added ethidium bromide.

Estimates of DNA accessibility in chromatin range from 28%, with acridine orange as the donor to 60%, with the bisbenzimidazole dye 33258 Hoechst (Table 1). This range may reflect variations both in the correlation of donor and acceptor binding distributions and in the quantum yield of donor molecules bound to different DNA sequences. Differences in the mode of binding of donor molecules might also be important. Quinacrine and acridine orange intercalate between DNA bases¹⁴, while 33258 Hoechst, which is a large bisbenzimidazole dye, may occupy one of the grooves of the DNA double helix¹⁷. Preliminary measurements indicate that approximately half of the sites in DNA for 33258 Hoechst binding are available in calf thymus chromatin. If some of these sites are inaccessible to ethidium bromide, the above value of 60% for DNA accessibility in chromatin obtained with 33258 Hoechst as energy donor would be an overestimate. The figures for DNA availability reported here bracket results from experiments involving polylysine or direct measurements of dye binding³⁻⁸. Similar estimates for DNA accessibility in chromatin, using

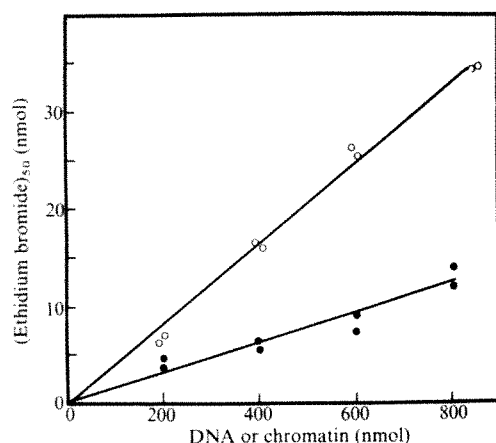


Fig. 2 Energy transfer titration of available phosphate in calf thymus DNA or chromatin. The amount of ethidium bromide at which quinacrine fluorescence is reduced 50%, obtained from data such as those of Fig. 1, is plotted against the amount of calf thymus DNA (○) or calf thymus chromatin (●) present in solution. Data were obtained at ratios of ethidium to phosphate of 0.025 or less. Calf thymus chromatin was prepared by extracting a crude nuclear pellet with 0.024 M EDTA, 0.075 M NaCl, pH 8.0¹⁶, swelling in 0.01 M NaCl, 0.005 M HEPES, pH 7.0, sedimenting through 1.7 M sucrose, and shearing at high speed.

measurements of energy transfer between other dye combinations, have been obtained by Gursky *et al.*¹⁸.

The differences in energy transfer between dye molecules bound to DNA and chromatin can arise if proteins simply exclude dye from some regions without affecting binding in

others¹⁹. They can also occur, however, if donor and acceptor molecules are statistically crowded into limited regions by correlated alterations in binding affinities. To determine whether the higher order structure of chromatin has such an influence on dye binding, energy transfer measurements were made on samples of chromatin either dissolved in 5 M urea or digested briefly with trypsin. Both treatments have been reported to disrupt chromatin structure, as determined principally by circular dichroic and hydrodynamic measurements, with at most a moderate dissociation of protein^{20,21}. The energy transfer between dyes bound to chromatin in 5 M urea was virtually identical to that with DNA (Table 1). Protein dissociation in 5 M urea, estimated by sucrose gradient centrifugation, was negligible. Following trypsin treatment which released less than 30% of the protein from DNA, the apparent accessibility of DNA in the chromatin increased to about three-quarters of that of free DNA (Table 1). Both urea and trypsin treatments increase the apparent accessibility of chromatin to ethidium bromide more than they alter the ratio of protein to DNA. These results are consistent with the idea that reaction of dyes with chromatin depends on chromatin conformation as well as on the literal coverage of chromosomal DNA by protein. It cannot, however, be excluded that a change in the mode of interaction of the residual protein with DNA contributed to our observations.

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Table 1 Estimation of DNA exposure in chromatin using electronic excitation energy transfer

Energy donor	Polymer	(Ethidium bromide) ₅₀ /Pi [‡]	DNA accessibility§
Quinacrine	DNA	0.044	1.00
	Chromatin	0.016	0.36
	Trypsinised*	0.036	0.83
	Chromatin		
Acridine orange	DNA	0.027	1.00
	Chromatin	0.008	0.28
	Trypsinised*	0.019	0.70
	Chromatin		
	DNA in 5 M urea†	0.024	1.00
33258 Hoechst	Chromatin in 5 M urea†	0.025	1.03
	DNA	0.041	1.00
	Chromatin	0.025	0.60
	Trypsinised*	0.032	0.78
	Chromatin		
	DNA in 5 M urea†	0.039	1.00
	Chromatin in 5 M urea†	0.040	1.03

All measurements were made in 0.01 M NaCl, 0.005 M HEPES, pH 7.0, 21–22°C. DNA and chromatin concentrations were between 1×10^{-4} and 4×10^{-4} M. Energy donor concentrations were between 1×10^{-7} and 7×10^{-7} M. 33258 Hoechst (2-(2-(4-hydroxyphenyl)-6-benzimidazolyl)-6-(1-methyl-4-piperazyl)-(benzimidazol-2HCl)) was a gift of Dr H. Loewe, Hoechst AG, Frankfurt.

* Chromatin was treated with 8U of tosylphenylchloroketone-treated trypsin (Worthington) per μ mol chromatin DNA phosphate for 2 h at 22°C before measurements were made.

† Urea was added to a final concentration of 5 M in 0.01 M NaCl, 0.005 M HEPES, pH 7.0. Addition of ethidium bromide to DNA plus quinacrine in this solvent did not quench quinacrine fluorescence.

‡ (Ethidium bromide)₅₀/Pi is the estimated ethidium bromide/phosphate saturation of DNA or chromatin at which donor fluorescence is quenched by 50%. In most experiments, this was determined by fitting data like those of Fig. 1 to a straight line after the addition of 8–10 nmol of ethidium bromide. When acridine orange was the energy donor, however, such plots consistently exhibited upward concavity, and initial slopes were estimated using least squares fits of data after the addition of only approximately 5 nmol of ethidium bromide.

§ DNA accessibility is calculated as the ratio of (ethidium bromide)₅₀/Pi in chromatin relative to that with DNA under the same conditions.

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Concanavalin A cap formation on polymorphonuclear leukocytes of normal and beige (Chediak-Higashi) mice

TREATMENT of a variety of cell types with concanavalin A (con A) leads to the aggregation of the lectin into one region or pole of the cell, forming a so-called cap. Not all cells cap (erythrocytes and normal cultured fibroblasts do not). Cells which do cap can be distinguished as (a) lymphocytes¹ and rabbit exudate polymorphonuclear leukocytes (PMN) (our data) that cap directly when incubated with low concentrations of con A at 37°C, and (b) cells exemplified by SV40-transformed fibroblasts² and lymphocytes incubated with high (more than $5 \mu\text{g ml}^{-1}$) concentrations of con A¹ that cap only after incubation with colchicine which

Table 1 Distribution of FITC-con A on PMN from normal and beige (CH) mice

Line	Preincubation	Postincubation	Normal (%)			CH (%)		
			Random	Capped	Patchy	Random	Capped	Patchy
1	Buffer	None	62	11	27	33	44	23
2	Colchicine (10^{-6} M)	None	23	55	22	32	50	19
3	Lumicolchicine (10^{-6} M)	None	73	11	16	—	—	—
4	Cyclic GMP (0.5×10^{-4} M)	None	70	8	22	76	10	14
5	Carbachol (10^{-6} M)	None	87	2	11	72	8	20
6	PMA (10 ng ml $^{-1}$)	None	77	6	17	69	6	25
7	Guanine (0.5×10^{-4} M)	None	—	—	—	25	46	29
8	Guanosine (0.5×10^{-4} M)	None	—	—	—	30	46	24
9	GMP (0.5×10^{-4} M)	None	71	9	20	35	47	18
10	Cyclic AMP (10^{-3} M) + theophylline (10^{-3} M)	None	67	8	25	41	38	24
11	Cyclic GMP (0.5×10^{-4} M) + colchicine (10^{-6} M)	None	72	12	16	73	5	22
12	Carbachol (10^{-6} M) + colchicine (10^{-6} M)	None	76	10	14	—	—	—
13	PMA (10 ng ml $^{-1}$) + colchicine (10^{-6} M)	None	75	14	11	—	—	—
14	Buffer	Buffer	70	10	20	—	—	—
15	Buffer	Colchicine (10^{-6} M)	43	38	19	—	—	—
16	Buffer	Cyclic GMP (0.5×10^{-4} M) + colchicine (10^{-6} M)	65	15	20	—	—	—
17	Cyclic GMP (0.5×10^{-4} M)	Colchicine (10^{-6} M)	45	33	22	—	—	—

Cell monolayers were preincubated at 37° C in modified Hanks medium containing 5 mM glucose plus the drugs indicated. After 30 min, fresh glucose-free medium containing FITC-con A ($5 \mu\text{g ml}^{-1}$) plus the same concentration of drugs was added and incubation was continued for 15 min. Cells were rinsed and either fixed immediately or postincubated for 20 min at 37° C with medium containing drugs before fixation. Results were obtained by examining 100 cells at random and assigning each cell to one of the 3 labelling categories (see text) according to the distribution of fluorescence. All experiments were done using blood from one normal and one CH mouse (3–5 weeks old) and with the same solutions of drugs and FITC-con A. The data in lines 1–13 are results from one representative experiment and those in lines 14–17 from a separate experiment. Some variation was observed between blood obtained from different animals, but qualitatively all effects were the same. The range of capping in the absence of drugs varied between 8 and 19% in 11 normal mice and between 36 and 58% in 11 CH mice. In all experiments, about 20% of cells showed a patchy labelling with FITC-con A.

disrupts microtubules (MT). These differences between cell types are not understood.

We report here that peripheral blood PMN from normal mice (C57/6J $+/+$ and $+/bg$) do not cap with con A except after colchicine treatment. This colchicine-induced capping is antagonised by cyclic GMP and agents that stimulate its production. In addition, we show that PMN from beige or Chediak-Higashi (CH) mice (C57/6J bg/bg) cap spontaneously with con A and that capping on these cells is also blocked by cyclic GMP. These results suggest a role for cyclic GMP in the regulation of surface phenomena that depend on MT and form the basis for a new hypothesis to explain the pathogenesis of the CH syndrome.

Mouse peripheral blood PMN were obtained as before³ and suspended in modified Hanks medium containing 5 mM glucose. Fluorescein-conjugated con A was prepared by incubating fluorescein isothiocyanate (FITC, Sigma) at a concentration of 0.05 mg mg^{-1} con A for 1 h at 0° C in the presence of 100 mM glucose (to protect the binding site) and phosphate buffer, pH 7.5, and purifying the product after dialysis by affinity chromatography on Sephadex G-50². Monolayers of cells were allowed to form on glass coverslips⁴ and labelled at 37° C with $5 \mu\text{g ml}^{-1}$ con A for 15 min, then rinsed in modified Hanks solution, fixed in 2% paraformaldehyde at 37° C for 10 min and wet mounted. The labelled cells were observed by combined phase-fluorescence using a Zeiss epifluorator with an FITC interference filter, 450 dichroic mirror, 53 barrier filter, and a 100 neofluar phase objective.

The observed fluorescence was categorised according to three labelling patterns: (1) random or diffuse; (2) cap—either a polar shell of fluorescence or a more concentrated knob or protrusion; (3) patchy—clumped aggregates—often perinuclear, or occasionally peripheral, or as larger more central irregular clusters. Examples of each labelling pattern can be seen in Fig. 1. Treatment of labelled cells with 50 mM α -methyl mannoside removed labelling of the first two patterns (diffuse or cap). Aggregates were not removed, however, indicating that the third or patchy pattern corresponds to endocytosed con A that is inaccessible to the

sugar. This endocytosed con A shows a superficial resemblance to surface (centrally located) caps. The development of the observed patterns seems to follow the sequence 1 to 2 to 3, or 1 to 3 directly (that is, endocytosis without an intermediate cap stage).

Table 1 is a summary of the patterns of fluorescence distribution observed with peripheral blood PMN from normal and CH mice. There was a striking increase in the proportion of capped cells and a corresponding decrease in the proportion of cells showing a random distribution of fluorescence in CH-PMN as compared with PMN from homozygous ($+/+$) or heterozygous ($+/bg$) normal mice (line 1). This difference is illustrated in Fig. 1a and b. Preincubation of monolayers for 30 min with colchicine promoted cap formation in normal PMN but had little additional effect on the extreme degree of capping in CH-PMN (lines 1 and 2). After colchicine treatment the degree of capping in normal PMN was very similar to that in untreated CH-PMN (compare Fig. 1b with c). Lumicolchicine (line 3), a photochemical derivative of colchicine that does not affect microtubules, did not affect capping.

The effects of cyclic nucleotides on capping were investigated because these compounds have been implicated in regulation of colchicine-sensitive (microtubular) functions in several systems. Thus both 3',5'-cyclic adenosine monophosphate (cyclic AMP) and compounds which increase its intracellular concentration, and colchicine, reduce antigen-induced histamine release⁵ and endocytosis-induced release of lysosomal enzymes⁶ from leukocytes. In contrast, both cyclic GMP and agents that elevate its intracellular concentration and deuterium oxide which favours MT assembly, augment histamine and enzyme release in the same *in vitro* models.

Our data show that preincubation of cell monolayers for 30 min with cyclic GMP (line 4) as well as carbamylcholine (carbachol; line 5) and phorbol myristate acetate (PMA, line 6) at concentrations which elevate cyclic GMP levels in PMN^{6,7} caused a marked reduction in capping on CH-PMN and slightly reduced capping on normal PMN. In fact, the degree of capping on CH-PMN after addition of

cyclic GMP was virtually identical to the untreated normal PMN (compare Fig. 1a with d). This effect was chemically specific for the cyclic guanine nucleotide since the potential products of its degradation, guanine (line 7), guanosine (line 8) and 5'-GMP (line 9) did not affect capping. In addition, cyclic AMP (line 10) had no significant effect on cap formation on either CH or normal cells.

When normal cells were preincubated with cyclic GMP (line 11), carbachol (line 12) or PMA (line 13) simultaneously with colchicine, the characteristic stimulation of capping by colchicine (compare line 2) did not occur (Fig. 1e and f). Thus cyclic GMP and compounds which increase intracellular concentrations of this nucleotide antagonised the extensive capping of con A that occurred on both untreated CH-PMN and colchicine-treated normal PMN. The effect of cyclic GMP was readily reversible, indicating that the observed effects occurred without loss of cell viability. Cap formation on normal PMN was enhanced by incubation with colchicine both before (line 2) and after (line 15) labelling with FITC-con A and was antagonised by the simultaneous presence of cyclic GMP in both cases (lines 11 and 16). Cells incubated with cyclic GMP before labelling and with colchicine after labelling (line 17), however, showed a similar increase in con A capping to cells exposed to colchicine alone.

Two mechanisms have been suggested to explain the effect of colchicine on cap formation. According to one hypothesis¹, high concentrations of con A induce an association between intracellular colchicine-binding proteins (presumably MT) and the cell surface that inhibits the lateral migration of bound ligands within the membrane, and colchicine, by virtue of its effects on MT, permits

this movement of surface constituents to resume. A second hypothesis^{8,9} is that cell or membrane movements are required to aggregate lectin-receptor complexes: con A immobilises the cell and colchicine promotes capping only under conditions in which it can restore cell movement. We have shown that cyclic GMP, which stimulates both random and directed (chemotactic) cell movement⁷ does not enhance capping by con A on normal PMN. Further, cyclic GMP antagonises the increase in capping that is induced by colchicine in normal PMN. These observations are consistent with the first hypothesis, in which capping is independent of cell movement.

We have also shown increased capping by con A on PMN from CH mouse. This mutant^{10,11}, like mutants of mink¹² and cattle¹³, is a homologue of the Chediak-Higashi syndrome of man¹⁴, a rare autosomal recessive disorder characterised by partial albinism, the presence of giant granules in most granule-containing cells, impaired leukocyte chemotaxis and defective lysosomal degranulation. The current view of the CH syndrome is that subcellular granule membranes may be defective in structure or function^{15,16}. The apparent increase in the mobility of lectin-receptor complexes on the plasma membranes of PMN from CH mice, which results in extensive cap formation, indicates that this view should now be extended to include other membranes. Furthermore, membranes of CH-PMN behave like membranes of colchicine-treated normal cells in which MT are depolymerised, in terms of their capping response to con A. This could show that the fundamental defect may be an abnormality in MT polymerisation and/or in the interaction between membranes and MT.

The intracellular mechanisms that regulate MT assembly are not yet understood. Our observation, however, that cyclic GMP antagonises the increased con A capping due to colchicine in normal PMN suggests that the cyclic nucleotide may promote MT polymerisation. Reports that cyclic GMP levels increase in lymphocytes¹⁷ and human PMN (J.M.O. and R.B.Z., unpublished) after binding of con A and that the number of MT seen by electron microscopy in human PMN is increased by agents that elevate cyclic GMP¹⁸ are consistent with this proposal. We suggest that in normal PMN binding of con A may elevate cyclic GMP and thereby promote MT assembly. In CH-PMN the generation of cyclic GMP in response to con A may be inappropriately low.

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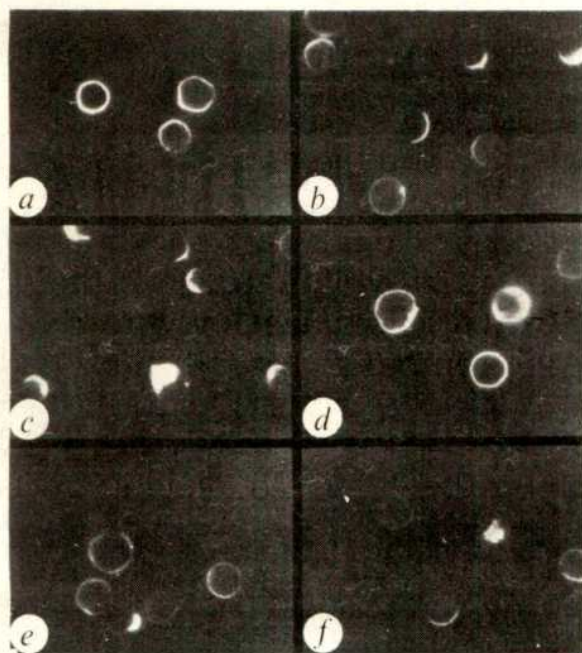


Fig. 1 The distribution of FITC-con A on peripheral blood PMN from normal and beige (CH) mice. *a*, Normal PMN: the diffuse labelling pattern predominates. *b*, CH-PMN: most cells are capped. *c*, Normal PMN incubated with $1 \mu\text{M}$ colchicine: the degree of capping is similar to CH-PMN without colchicine. In one cell the cap has begun to be internalised and fluorescence is seen in a clumpy aggregate. *d*, CH-PMN incubated with $0.5 \times 10^{-4} \text{M}$ cyclic GMP: the labelling pattern is diffuse as in the normal PMN. *e*, Normal PMN incubated with both cyclic GMP and colchicine: cyclic GMP antagonises the increased cap formation seen with colchicine alone. *f*, CH-PMN incubated with both cyclic GMP and colchicine: most of the label is distributed randomly. One cell shows a central cluster of fluorescence that resembles a central cap. This fluorescence cannot, however, be removed by α -methylmannoside and so it most likely represents internalised con A.

Specific IgE antibodies in immune adherence of normal macrophages to *Schistosoma mansoni* schistosomules

PARASITIC helminths characteristically provoke high levels of IgE antibodies in man and similar reagin-type antibodies in animals. But it has not been demonstrated conclusively that these antibodies have a protective role.

When they are incubated in the serum of Fisher rats immune to *Schistosoma mansoni*, the peritoneal adherent cells of normal inbred Fisher rats are strongly adherent to *S. mansoni* schistosomules maintained *in vitro*^{1,2}. Electron microscopy has shown that the adherent cells are macrophages. Here we report experiments which indicate that the factors in immune serum responsible for the macrophage adhesion are specific anti-schistosome IgE antibodies.

Serum samples were obtained by bleeding infected Fisher rats at various intervals after exposure to 400 cercariae of *S. mansoni* (group 1). Uninfected Fisher rats were used as a control (group 2). A highly significant correlation was obtained between the percentage of macrophage-coated schistosomules and the time elapsed since infection ($r=0.562$; $n=80$; $P<0.001$).

After infection for 30 d, the mean percentage of macrophage-coated schistosomules was significantly higher in group 1 than in group 2 (Fig. 1). After 50 d, the mean percentage of macrophage-coated schistosomules was 88.4% (s.d. 9.4; $n=8$) compared with a value for the uninfected control sera of 29.8% (s.d. 23.1; $n=6$; $P<0.001$). The control values could be reduced to a mean of 10% if sera from uninfected germ-free rats were used (A.C., J.-P.D., M.C., and H.B., unpublished).

Adherence of immune serum-incubated macrophages was inhibited when the serum was heated at 56°C for 3 h and

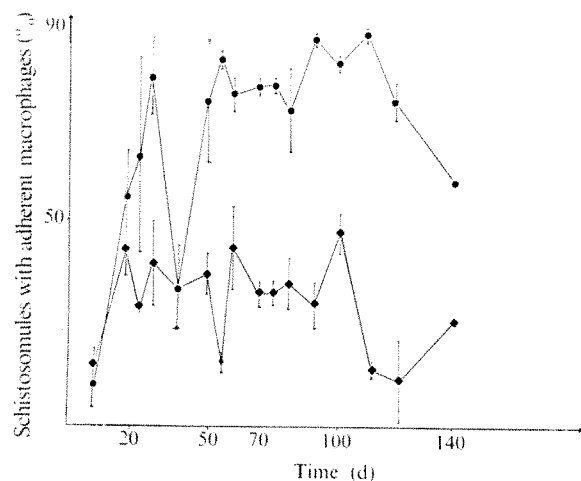


Fig. 1 Relationship between the adherence of serum-incubated macrophages and the period of infection of rats by *S. mansoni* cercariae. Normal syngenic Fisher rat macrophages were collected by peritoneal washing with Eagle's MEM and plated in Falcon dishes. Non-adherent cells were washed off after 3 h incubation. Adherent population was incubated in MEM containing 20% of either immune (group 1) or normal (group 2) rat serum. At least five replicates in each group were performed. Approximately 200 *S. mansoni* schistosomules recovered according to the technique described by Clegg and Smithers³, were added and adherence of macrophages was estimated after 3 h contact under an inverted phase contrast microscope. More than 30 adherent macrophages to one schistosomule was considered to be significant and the percentage of embedded schistosomules was calculated. ●, Group 1; ◆, group 2; s.d. are indicated by vertical lines through points. Statistical analysis (Student's *t* test and Wilcoxon two samples test) showed highly significant ($P<0.01$) differences between the two groups.

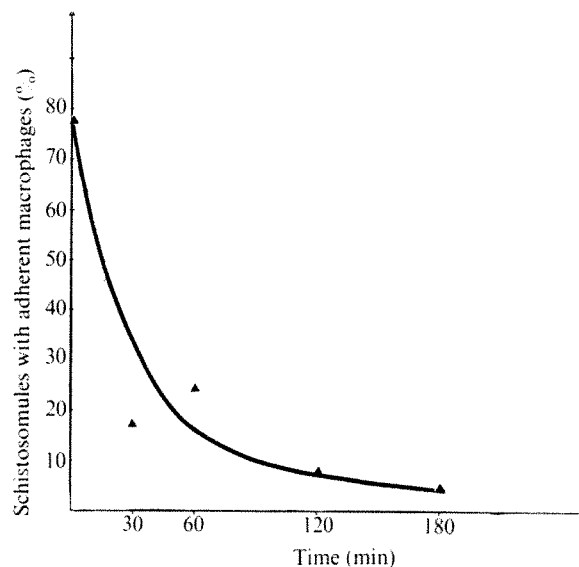


Fig. 2 Thermolability of serum factor involved in macrophage adherence to *S. mansoni* schistosomules, as estimated by heating infected rat serum 30–180 min at 56°C before incubation with normal macrophages and schistosomules. Results are expressed as percentage of schistosomules with adherent macrophages.

was not restored by addition of guinea pig fresh serum. It was therefore assumed that a thermolabile factor could be responsible for macrophage adherence.

Absorption of rat immune sera by *S. mansoni* soluble antigens before incubation was shown to decrease significantly the adherence of the macrophage to the schistosomules, indicating that the factor was probably a thermolabile antibody. To assess this hypothesis, various experiments were devised.

The thermolability of the serum factor was confirmed by estimating the percentage of macrophage-coated schistosomules after incubation with sera heated for periods of 30–180 min (Fig. 2). Absorption of rat *S. mansoni* immune sera by increased quantities of goat anti-rat IgE serum, specific for ϵ chain, or $F(ab')_2$ of the same antiserum⁴, led

Table 1 Comparison of immune adherence of normal macrophages incubated in infected-rat serum before and after absorption with anti-rat IgE immunosorbent

Serum	% Schistosomules with adherent macrophages (mean \pm s.d.)	Significance	IgE level (μ g ml ⁻¹) (mean \pm s.d.)
Unabsorbed	86.25 \pm 6.02	$C_1 = 3.26$ $P \leq 0.01$	9.70 \pm 0.47
Day 59			
Absorbed	4.80 \pm 3.56	$t = 25.455$ $P < 0.0001$	1.31 \pm 0.32
Unabsorbed	92.25 \pm 5.06	$C_1 = 3.26$ $P \leq 0.01$	10.20 \pm 0.44
Day 68			
Absorbed	12.2 \pm 9.70	$t = 29.371$ $P < 0.0001$	0.01 \pm 0.40

The solid phase anti-IgE was prepared with goat-anti-rat IgE $F(ab')_2$ (ϵ specific) covalently linked to cyanogen bromide activated Sepharose. The rat serum was absorbed out by passage through the column, concentrated to the original volume and incubated with the normal rat macrophages. Means and s.d. of the percentages of coated schistosomules were calculated. Serum IgE determination was carried out by a radio-immunoassay; radioactivity bound was precipitated by 33% ammonium sulphate⁵.

to a progressive decrease of the macrophage adherence (Fig. 3).

Similar experiments with anti-rat IgG (IgG₁, IgG_{2a}, IgG_{2b}), IgM and IgA goat serum⁵ did not exhibit any significant decrease.

To avoid possible interference by soluble IgE-anti-IgE complexes and excess of anti-IgE antibodies, the same absorption experiment was carried out in solid phase using goat IgG anti-rat IgE linked to Cyanogen Bromide-activated Sepharose 3B. In these experimental conditions, a highly significant decrease of macrophage-coated schistosomules was observed (Table 1); the disappearance of IgE was controlled by radio-immunoassay.

The involvement of specific IgE antibodies in the immune adherence of normal macrophages to *S. mansoni* schistosomules could therefore be inferred. Further controls of specificity were performed with IgE myeloma serum (IR 162) (ref. 7) and with sera containing unrelated reaginic antibodies: anti-ragweed, anti-ovalbumin, anti-BGG-DNP and anti-*Nippostrongylus brasiliensis* with respective homologous PCA titres of 1:512, 1:256, 1:512, and 1:512, according to the technique of Jarrett and Ferguson⁸. None of these sera elicited any significant immune adherence of macrophages to schistosomules.

To investigate the mechanism of action of IgE in immune adherence, the possibility of nonspecific binding of IgE molecules on the macrophage surface was considered. Three different techniques were used. Rosette formation was obtained when normal rat macrophages incubated with rat IgE myeloma protein were put into contact with specific anti-rat IgE linked by glutaraldehyde on group O, rhesus negative, human red cells. Similar results were obtained when macrophages were incubated in serum from rats infected with *S. mansoni*.

Macrophages incubated with these immune rat sera demonstrated a fluorescent surface staining with goat serum anti-rat IgE revealed by anti-goat fluorescein-labelled globulins. No staining was obtained when the anti-*S. mansoni* rat serum was previously heated (56°C for 3 h) or absorbed by F(ab')₂ anti-rat IgE. The binding of IgE to the macrophage surfaces was confirmed by the use of peroxidase-labelled IgG anti-rat IgE and an ultrastructural study.

It seemed, therefore, that the nonspecific binding of IgE on rat macrophages resembles the cytophilic binding of some immunoglobulin classes in man and mouse^{9,10}. In our model the immune-adherence of the macrophage to schistosomules therefore seems to be the result on the one hand, of the specificity of IgE antibody against the parasite and, on the other hand, of a nonspecific binding on the macrophage surface, probably by the constant part of the IgE molecule.

Preliminary observations have indicated that the immune adherence of macrophages described here may have some importance in the killing of *S. mansoni* schistosomules *in vitro* methods¹¹, is highly accelerated if specific IgE antibodies for *S. mansoni* schistosomules, as studied by *in vitro* methods¹¹, is highly accelerated if specific IgE mediated adherence of normal macrophages occurs at the same time. Implications of these observations might be considered at two levels.

In the rat, reaginic antibodies were reported and their possible significance in *S. mansoni* infection considered^{12,13}. Our present observations bring an indication of a positive function of IgE antibodies in immunity to schistosomes in the rat.

Increased levels of IgE have been reported for a number of helminth infections in man¹⁴ and specific human IgE antibodies against schistosomes have now been demonstrated (J.-P.D., *et al.*, unpublished). It is reasonable then to consider the possible role of IgE-mediated macrophage adherence in schistosomiasis and other helminthoses of man.

To our knowledge, this is the first time that IgE antibodies

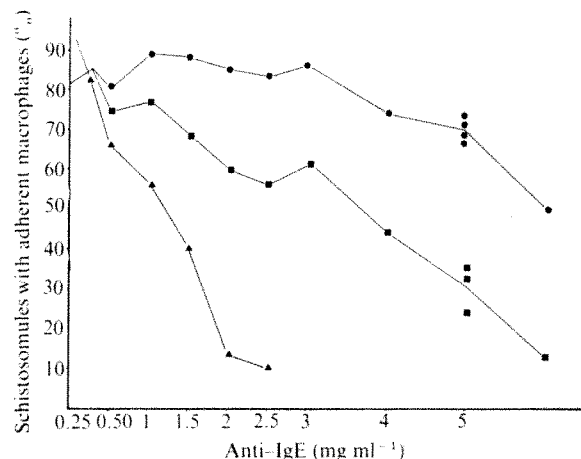


Fig. 3 Effect of absorption by increasing quantities of anti-rat IgE on adherence of incubated macrophages to *S. mansoni* schistosomules. A pool of group 1 rat sera was incubated with different anti-IgE concentrations (mg ml⁻¹), 2 h at 37°C, overnight at 4°C and centrifuged. Macrophages were incubated in supernatants. Δ, Absorption by goat IgG anti-rat IgE (ε specific); ■, absorption by F(ab')₂ prepared from goat IgG anti-rat IgE; ●, control experiment with F(ab')₂ prepared from normal goat IgG.

have been implicated in macrophage adherence reactions and the phenomenon should be investigated in other situations where specific IgE antibodies are involved. In any event, our experiments indicate that specific IgE anti-schistosome antibodies may have a central role in the immunity of the rat to this parasite, and are not merely concerned in the concurrent development of immediate hypersensitivity.

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T-cell response to polyvinyl pyrrolidone is linked to maturity of B cells

HUMORAL antibody responses differ in their thymus dependency. Some antigens such as heterologous proteins¹, heterologous erythrocytes¹ and transplantation antigens² show a pronounced requirement for T helper cells, whereas other antigens such as bacterial polysaccharides^{3,4}, polymerised flagellin⁵ and certain synthetic polymers^{4,6} can induce a humoral antibody in the B lymphocytes in the absence of T helper cells. It has been suggested that the thymus-independent antigens are able to trigger the B cells directly because of their polymeric structure with repeating antigenic determinants⁷, because of their mitogenic properties for B cells⁸, or because they have the capacity to activate complement by means of an alternative pathway thus providing the C3 receptor-bearing B cells with an extra, activating signal⁹. It has also been proposed that thymus independency is only a relative, quantitative phenomenon and that some antigens seem thymus-independent since they need only extremely few T cells for interaction and triggering of the B cells¹⁰. It has also been demonstrated that thymus-independent antigens are slowly metabolised and non-degradable in the body, in contrast to the thymus-dependent ones⁶. The significance of this last observation is not known. We report some properties of the humoral antibody responses to polyvinyl pyrrolidone in mice. The thymus dependency of this response⁶ is shown to be a function of the stage of maturation of the B lymphocytes.

Inbred mice of (A/Sn×C57BL)F₁ genotype 8–12 months old were used as recipients in cell transfer experiments; animals at ages indicated below were used as cell donors. Immunisation with polyvinyl pyrrolidone (PVP; molecular weight 360,000) was carried out as previously described¹ and serum antibody was determined by a haemolysis of PVP-labelled erythrocytes¹. Preparation of cell suspensions and irradiation of mice was as previously described¹.

Spleen cells from adult mice (2 months old) and young mice (2 weeks old) were compared with regard to their capacity to mount a humoral antibody response to PVP after transfer to lethally irradiated recipient mice. Table 1 shows that the adult spleen cells were able to produce antibody to this antigen, and that the addition of normal adult thymus cells markedly suppressed the antibody response. In contrast, we found that the young spleen cells produced only small amounts of antibody by themselves, the antibody response being enhanced by the addition of thymus cells from normal adult mice. The effects observed on the antibody production in the PVP system were the result of T cells residing in the cortisone-resistant part of the thymus,

Table 1 Helper or suppressor effect of thymus cells exerted on young or adult spleen B cells in their response to PVP

Spleen	Thymus*	Antibody response†
2 months, 2×10^7	—	3.9 ± 0.5
2 months, 2×10^7	10^7	1.8 ± 0.5
2 weeks, 2×10^7	—	2.2 ± 0.5
2 weeks, 2×10^7	10^7	4.1 ± 0.3

*Normal, 2 month old mice were used as thymus cell donors.

†Humoral antibody titre (\log_2) 2 weeks after lethal irradiation with 750 rad and intravenous infusion of cells together with 0.1 μ g PVP mixed with the cells. Each figure represents the mean from 18 animals \pm s.e.m.

Table 2 Helper and suppressor effects of thymus cells. A comparison between thymus cells from normal, immune and tolerant animals

Thymus	Antibody titre*	
	Adult spleen	Young spleen
Normal	1.2 ± 1.2	4.5 ± 0.3
Immune†	1.9 ± 0.7	3.3 ± 0.4
Tolerant‡	5.6 ± 0.2	1.8 ± 0.9
No thymus cells given	5.6 ± 0.3	1.8 ± 0.8

*Humoral antibody titre (\log_2) in lethally irradiated mice 2 weeks after 750 rad together with intravenous infusion of the indicated cells with 0.1 μ g PVP. Each figure is the mean of five to six animals \pm s.e. Animals had received 2×10^7 spleen cells, young or adult, either alone (as in the bottom group), or together with normal, immune or tolerant thymus cells in a dose of 10^7 .

†Immune thymus cell donors had received 0.1 μ g PVP 1 month earlier as an intraperitoneal injection.

‡Tolerant thymus cell donors had received 1,000 μ g PVP intraperitoneally 1 month earlier.

as the activity of the thymus cells was not affected by passage over anti-Ig columns, and the remaining thymus cells after cortisone treatment of the donor mice were still active (B. A. and H. B., unpublished). Thymus cells treated with mitomycin C were inactive, indicating that living T cells were needed for the effects (B. A. and H. B., unpublished). Comparing the spleen B cells from young and adult mice we found the same percentage (50–60%) of cells bearing Ig on their surface but the young spleen cells had only 2–17% C3 receptor-bearing cells compared to 21–56% in the adult spleens. This possibly indicates a role for the C3 receptor in determining thymus dependency. Depletion

Table 3 Maturing B cells from the bone marrow can be helped in anti-PVP antibody response by specifically immune thymus cells

Bone marrow	Thymus*	Antibody titre†
2×10^7	—	0.2 ± 0.2
2×10^7	10^7 normal	1.2 ± 1.2
2×10^7	10^7 tolerant	1.3 ± 0.6
2×10^7	10^7 immune	4.6 ± 0.4

*Normal, tolerant and immune thymus had received the same treatment as corresponding cell donor mice in Table 2.

†Humoral antibody titres in lethally irradiated mice (750 rad) receiving the indicated cells intravenously together with 0.1 μ g PVP. Animals were bled 11 days after irradiation and cell transfer. Each group consisted of five to ten mice.

of C3-bearing cells by EAC columns¹¹ or EAC rosette sedimentation¹² and further studies on the thymus dependency in the depleted populations may provide the answer.

The effects of thymus cells on the humoral antibody response to PVP could be specific, mediated by T lymphocytes recognising antigenic determinants on the PVP molecules and acting as specific helper or suppressor cells. The effects of the thymus cells could, however, also be nonspecific, mediated, for example, by the release of factors inhibiting or stimulating the rate of proliferation and/or maturation of the B cells. To discriminate between these possibilities, the activities of thymus cells from normal, immune and tolerant donor mice were compared. Table 2 shows that pretreatment of the thymus donor mice with high doses of PVP, capable of producing tolerance, abolishes the suppressing and the helping activities of the thymus cells, whereas normal and immune thymus cells exerted both suppressing and helping activities. Table 3 shows that immune thymus cells actually perform better in one respect

than normal and tolerant thymus cells, namely in their capacity to act as helper cells for maturing B cells early after lethal irradiation and bone marrow reconstitution.

Table 1 shows that, when 2×10^7 spleen cells and 10^7 thymus cells are given, young spleen cells are stimulated and adult spleen cells are suppressed in their anti-PVP response. We point out, however, that these effects are observed only at certain critical ratios between spleen cells and thymus cells. Table 4 shows that the suppressing effect of thymus cells is not present when lower or higher doses of thymus cells than 10^7 are used to modify the response of 2×10^7 adult spleen cells. One possibility is that stimulation and suppression are exerted by different subpopulations of immunocompetent cells present in the thymus, and that at high cell doses the suppressor cell activity is counteracted by helper cells. It may also be that heterogeneity exists in the adult spleen B cell population and that subpopulations

of immature B cells and therefore the effects may be of the same kind as those we report.

It is still not known whether the enhancing and suppressing activities of T cells on antigens like PVP are mediated by the same or by different subsets of T cells or thymus cells. In either case, the possibility of detecting helper cell activity or suppressor cell activity, evident from our data, depends on the stage of maturation of the B cells used in the experiments.

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Table 4 Helper and suppressor effects of thymus cells: variations with cell dose

Normal thymus	Antibody titre*	
	Adult spleen	Young spleen
—	3.8 ± 0.2	1.0 ± 0.5
4×10^7	2.6 ± 0.7	0.9 ± 0.6
2×10^7	4.0 ± 0.0	1.0 ± 0.5
10^7	$0.0 \pm (< 1.0)$	3.4 ± 0.3
5×10^6	4.4 ± 0.2	3.7 ± 0.2
2×10^6	4.2 ± 0.5	3.8 ± 0.4
10^6	3.3 ± 0.8	ND

*Experimental conditions as in Table 2. For adult spleen, five recipient mice per group; for young spleen, eight to ten mice per group. ND, not determined.

of B cells with different requirements for helper T cells exist. The young spleen cells can be stimulated by relatively small doses of thymus cells and when the thymus cell dose is increased over a critical level (10^7 thymus cells for 2×10^7 young spleen cells) suppression is taking place. This could be due to heterogeneity of thymus cells, that is, different subsets of cells exerting suppression and stimulation. One population of T cells exerting both functions but effective at different optimal concentrations would give the same picture.

The humoral antibody response to PVP was found to be thymus-independent in adult thymectomised, lethally irradiated and bone marrow-reconstitution mice 3–4 weeks after the reconstitution, when presumably the B cells were as mature as those of normal adult mice⁴. We would, therefore, like to modify the concept of thymus independence for this antigen in the following way. Mature B cells respond to PVP without T-cell help. Under certain experimental conditions T cells may instead suppress the response, as evidenced from numerous publications of suppressor T cells during the past few years in a variety of systems¹³ including the anti-PVP system of mice^{14–16}. Immature B cells from young mice require T cells as helpers to respond optimally to PVP. There is at least one previous publication indicating that the concept of T cell dependency in immature B cells may be a more general phenomenon: it has been reported that the B cells from Peyer's patches in mice are poorly stimulated by the B-cell mitogen *E. coli* lipopolysaccharide¹⁷ and that the mitogenic response is enhanced by the addition of T cells. The Peyer's patches may contain a high propor-

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Absence of age-resistance in neonatally thymectomised chickens as evidence for cell-mediated immune surveillance in Marek's disease

MAREK's disease virus (MDV) of chickens induces lymphomas and other lymphoproliferative changes, which, depending on the virulence of the strain used, may terminate in paralysis and death. Chickens genetically selected for resistance, however, are refractory to clinical MD and most chickens that are fully susceptible when young develop resistance as they become older. Resistance acquired with age is expressed through lesion regression^{1,2}. Our studies with genetic and age resistance established that humoral immunity did not play a major role in either type of resistance^{3,4} and now we present evidence that the thymus-dependent immune system is of principal importance in the natural resistance of old chickens.

Two experiments were conducted. White Leghorn chickens of cross 15×7 with maternal MDV antibody were either thymectomised (TX) or TX and treated with cyclophosphamide (CY, Cytosan, Mead Johnson Laboratories, Evansville, Indiana) at hatching. These and appropriate control groups were raised in isolation until 8 weeks old; and then inoculated intra-abdominally with cell-associated MDV (JM strain). Before virus inoculation, all groups were tested⁵ and found to be free from adventitious infection with MDV. Chickens were observed for 12 and 14 weeks after inoculation, in the first and second experiments respectively. Chickens dying during the observation period and those surviving at the termination of the experiments were necropsied and if gross lesions of MD were absent, peripheral nerves and gonads were examined for histological lesions⁶. The survivors were also tested for MDV antibody by the agar gel precipitin test. In TX groups, only the chickens that lacked grossly detectable thymus remnants were considered adequately TX and included in the data.

Table 1 T-cell and B-cell functions in chickens thymectomised (TX) and thymectomised plus cyclophosphamide (CY) treated at hatching (experiment 2)

Treatment at hatching	No. of chickens	Thymus remnants	Delayed hypersensitivity +/tested	Sheep erythrocytes (Haemagglutination test) +/tested†	Mean titre (log ₂)‡	Antibody to* <i>B. abortus</i> (Plate agglutination test)		MDV (agar gel precipitin test) +/tested
						+/tested	Mean titre (log ₂)	
γ-radiation	16	Present	15/16	15/15	6.9	15/15	2.5	16/16
TX + γ-radiation	9	Absent	4/9	9/9	7.4	9/9	3.4	2/2
TX + γ-radiation + CY	11	Absent	3/11	0/11	0.0	0/11	0.0	0/2
CY treated	15	Present	15/15	10/15§	3.7	1/15	3.0	0/14
None	13	Present	13/13	13/13	8.8	12/13	1.8	13/13

* All groups were inoculated intravenously with sheep erythrocytes and *B. abortus* (2.5×10^8 cells of each) at 5 and 7 weeks of age and with MDV (intra-abdominally) at 8 weeks. Antibody to sheep erythrocytes and *B. abortus* was determined just before MDV inoculation. Tests for MDV antibody were done at the end of the experiment.

† Titre of $\geq 1:4$ was considered positive.

‡ Highest dilution tested was 1:512.

§ Five chickens negative by the haemagglutination test were also negative for antibody against *B. abortus*.

Chickens were thymectomised surgically⁵ within 30 h of hatching. On the day following surgery, TX and appropriate control chickens were exposed to 600 rad of total body γ radiation (88 rad min⁻¹ at 2 m from cobalt-60, 24,000 Ci, Michigan State University). Thymus function was tested by delayed-type hypersensitivity (DTH) reaction. Chickens were sensitised with complete Freund's adjuvant and 4 weeks later, tested for local reaction to intradermal injection of old tuberculin into the right wattle⁶.

Deficiency in the B-cell function was induced with 16 mg of CY given in four daily injections, each of 4 mg, commencing 2 d after hatching. In the CY-treated groups, only chickens that failed to produce antibody against sheep erythrocytes, *Brucella abortus* and MDV were included in the data (Table 1).

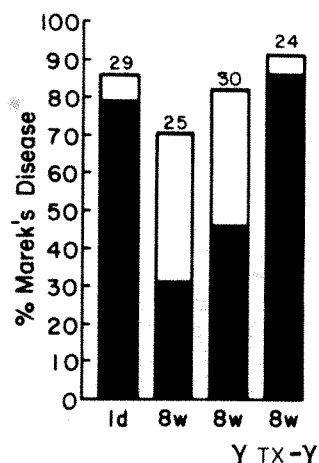
In the first experiment all survivors in MDV-inoculated but none in uninoculated control groups had MD antibody at termination. As Fig. 1 shows, the group exposed at 8 weeks had a significantly lower incidence of gross lesions and mortality than the group exposed at 1 d ($P < 0.01$, χ^2 test), thus demonstrating age resistance in the older chickens. As observed earlier^{1,2}, however, age resistance was not well pronounced at the level of microscopic lesions. The incidence of gross lesions and mortality in the γ-irradiated older group was slightly higher, although not significantly ($P > 0.5$), than that in untreated 8-week-old chickens. Like the unirradiated chickens, the irradiated chickens had a significantly lower incidence of gross lesions and mortality than that in the 1-d-old group

($P < 0.01$) thus illustrating the presence of age resistance. In the TX group, 24 chickens lacked thymus remnants at necropsy: 13 of these were negative for DTH. Among the 24 TX chickens, the incidence of mortality and gross lesions was 87%; significantly higher than that in the untreated ($P < 0.01$) and γ-irradiated 8-week-old groups ($P < 0.01$). In the second experiment (Fig. 2) the resistance of 8-week-old chickens was more pronounced than in the first. Incidence of death and gross lesions was 15%, whereas incidence in the 1-d-old group was 90%. γ Irradiation did not significantly influence the resistance of older birds. The thymus- and bursa-dependent functions of various treatment groups in the second experiment are given in Table 1. Of the 15 CY treated chickens, five were free of antibody against sheep erythrocytes and *B. abortus*. Four of the five were resistant, however, confirming that humoral immunity alone does not play an important role in age resistance to MDV. All 9 chickens in the TX and 11 in the TX + CY treated groups either died as a result of MD or had gross lesions at termination. These results were consistent with the results of the first experiment. Because CY treatment alone did not influence resistance, the lack of resistance in the TX + CY treated group was attributed to thymectomy. The incidence of microscopic lesions in older age groups was lower than in the first experiment probably because chickens were held longer and more lesions regressed.

The effect of the absence of T cells on MDV has not previously been reported, presumably because T-cell function cannot be completely obliterated with available surgical procedures. In our experiments also, thymectomy failed to eliminate all T-cell function because many TX birds, although lacking in detectable thymus remnants, reacted in DTH to old tuberculin. T-cell function was, however, definitely suppressed to significantly alter the MD response.

The results of this study also have important implications for the understanding of the pathogenesis of MD. The mechanism by which lymphoid cells are triggered to proliferate into lymphomas is not known. Because most of the lymphoma cells in MD are of T origin^{7,8} and because functional bursectomy does not influence lymphoma formation^{9,10}, it has been proposed that the target cell, that is the cell that undergoes neoplastic transformation, is of thymic origin. Several lymphoid cell lines have been developed from MD lymphomas¹¹⁻¹³ and the cells of these lines bear T-cell surface markers^{13,14}. In the light of this evidence for the T cell being the target cell for MD virus the high incidence of gross lesions in the TX birds in our experiments was unexpected. Our results can, however, be explained if, as stated above, neonatal thymectomy did not eliminate all T cells and the TX birds had enough remnant T cells to serve as the target for MDV, yet insufficient to mount a satisfactory cell-mediated immune response. The residual T cells may have originated from thymic remnants¹⁵ or from cells that had left the thymus before thymectomy. Nonetheless, our results indicate a pressing need for a more direct identification of the target cell in MD. Recognition of specific tumour

Fig. 1 The effect of neonatal thymectomy (TX) and γ-radiation (γ) on age resistance to MDV. Numbers below columns indicate the age in days (d) or weeks (w) at the time of MDV inoculation (1.0×10^4 PFU per chicken). Numbers above columns indicate the number of chickens in each group. Total MD includes chickens with histological lesions at termination of the experiment. Black indicates mortality plus gross lesions and white indicates total MD.



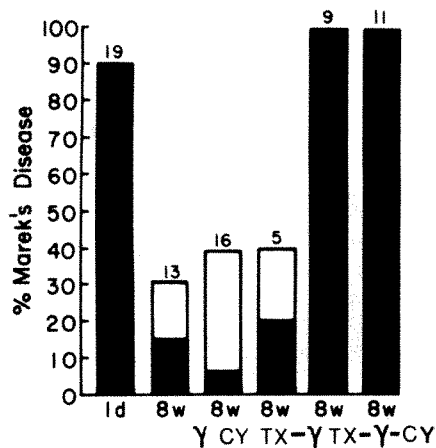


Fig. 2 The effect of neonatal thymectomy (TX) and γ -radiation (γ) and of TX- γ and cyclophosphamide (CY) treatment on age resistance to MDV inoculation (5.5×10^4 PFU per chicken). See legend to Fig. 1.

antigens¹³ may facilitate detection of transformation *in vivo*.

If T cells acted as target cells, the presence of the thymus was apparently not necessary for initial virus-cell interaction and for emergence of transformed clones. Because gross lesions were found in most chickens without thymus remnants, the initial transformation probably occurred in extra thymic T cells. This situation is in contrast with that of lymphoid leukosis in chickens where virus-induced transformation of target cells originates in B cells within the physical confines of the bursa of Fabricius and the removal of this organ, even well after many B cells have peripheralised, prevents tumour formation^{16,17}.

We concluded that age resistance and hence lesion regression in MD is mediated through thymus-dependent cellular immune functions.

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Effect of ascorbic acid on hyaluronidase inhibitor

THE presence of a physiological inhibitor of hyaluronidase (PHI) in mammalian serum was suggested by Duran-Reynals¹ who observed the destruction of spreading factor by blood. Hobby *et al.*² and McClean³ later reported the inhibition of microbial and mammalian hyaluronidase by serum. PHI, which is a glycoprotein and sensitive to heat⁴,

has been shown to increase in both bacterial and viral infections, cancer and various liver, kidney and rheumatic diseases^{4,5}. A small increase of PHI has been reported in scorbutic guinea pigs by Shack *et al.*⁶. These increases have been suggested^{5,8} to represent a defence against invasive microorganisms, or a non-specific response to tissue inflammation or destruction⁷. (Fischer-Szafarz⁷ described a hyaluronidase inhibitor in the serum of cancer patients which she believes is different from PHI.) Cameron and Pauling suggested that a high intake of ascorbic acid increases the biosynthesis of PHI, resulting in a decrease in hyaluronidase activity⁹. Consequently, the invasiveness of proliferative diseases would be prevented by reduced depolymerisation of the intercellular matrix of the proliferating cells. We have investigated the effect of doses of ascorbic acid of 0–1,000 mg kg⁻¹ d⁻¹ on levels of PHI in the serum of guinea pigs. Our results show that PHI is not affected by high levels of dietary ascorbic acid.

Guinea pigs receiving 20, 200, 400 and 1,000 mg kg⁻¹ d⁻¹ gained weight at identical rates throughout the experiment. Those animals which received no ascorbic acid lost weight after 14 d and developed scurvy (Fig. 1).

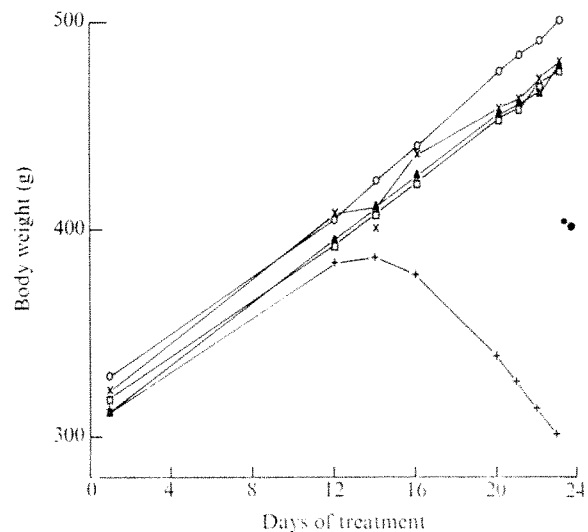


Fig. 1 Effect of ascorbic acid on body weight. Adult male Hartley guinea pigs were fed a vitamin C-deficient Reid Briggs diet and received varying amounts of ascorbic acid in drinking water for 22–23 d. Based on an average daily water consumption of 10 ml per 100 g body weight, the animals received 0, 20, 200, 400 or 1,000 mg kg⁻¹ d⁻¹. Body weights were measured periodically. +, Untreated; o, 20 mg kg⁻¹ d⁻¹; x, 200 mg kg⁻¹ d⁻¹; Δ, 400 mg kg⁻¹ d⁻¹; □, 1,000 mg kg⁻¹ d⁻¹. Each point represents the mean of 15 animals.

In experiment 1 the serum from each animal was assayed individually for PHI by the hyaluronidase turbidity assay (Table 1). Scorbutic guinea pigs had significantly more PHI ($P < 0.02$) than those receiving 20 mg kg⁻¹ d⁻¹ and there was no significant difference in levels of PHI among any of the groups receiving ascorbic acid. In experiment 2 (Table 1) sera from 15 animals were pooled in groups and assayed for PHI. Again the levels of PHI in the scorbutic group were significantly ($P < 0.01$) higher than in animals receiving ascorbic acid. Again there was no significant difference in PHI levels in the groups receiving ascorbic acid. Sera from animals in experiment 1 were then pooled and assayed for PHI using ³⁵S-chondroitin sulphate (ChS) as a substrate for hyaluronidase (Fig. 2). The greatest inhibition of hyaluronidase activity was achieved with sera of scorbutic animals. Animals receiving 200 or 400 mg kg⁻¹ d⁻¹ of ascorbic acid did not have a greater level of PHI than those receiving 20 mg kg⁻¹ d⁻¹.

Since scorbutic guinea pigs consume less food than normal animals, we investigated the effect of this decreased food

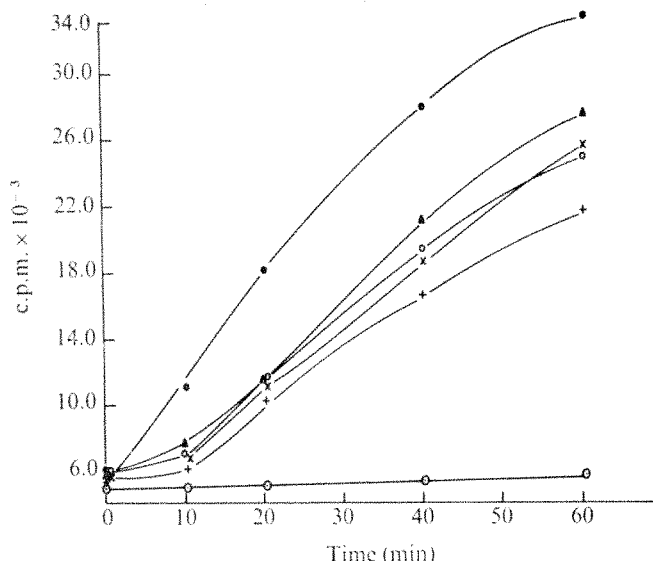


Fig. 2 Hydrolysis of ^{35}S -chondroitin sulphate by hyaluronidase. ●, Hyaluronidase control; +, hyaluronidase preincubated with sera from untreated animals; ○, hyaluronidase preincubated with sera from animals treated with 20 mg ascorbic acid $\text{kg}^{-1} \text{d}^{-1}$; x, hyaluronidase preincubated with sera from animals treated with 200 mg ascorbic acid $\text{kg}^{-1} \text{d}^{-1}$; ▲, hyaluronidase preincubated with sera from animals treated with 400 mg ascorbic acid $\text{kg}^{-1} \text{d}^{-1}$; ○, boiled hyaluronidase. The conditions for preincubation of the PHI and hyaluronidase were identical with those described for Table 1, except that 6.0 ml of enzyme was preincubated with 0.2 ml of guinea pig serum. After preincubation 3.0 ml of the hyaluronidase-PHI mixture was added to 3.0 ml of 0.3 M phosphate buffer, pH 5.5, containing 13 μg of ^{35}S -chondroitin sulphate (800,000 c.p.m.). The reaction was maintained at 37°C for 1 h. Samples (1 ml) were removed at the designated times and added to 1 ml of 2% cetylpyridinium chloride. After 2 h at room temperature, the precipitate was centrifuged, and 0.5 ml of supernatant was placed in 10 ml of Aquasol, and counted in a Packard liquid scintillation counter. The ^{35}S -chondroitin sulphate was isolated from costal cartilage which had been incubated in Krebs-Ringer bicarbonate containing 0.2 mCi of carrier free $\text{H}_2^{35}\text{SO}_4$.

Table 1 Effect of ascorbic acid on serum PHI

Ascorbate intake ($\text{mg kg}^{-1} \text{d}^{-1}$)	Experiment	Hepatic ascorbate ($\text{mg per 100 g tissue}$) ^{*†}	Inhibitor ^{*‡} (U)
0	1	1.43 ± 0.23	37.2 ± 11.2
	2	1.21 ± 0.59	40.8 ± 6.4
20	1	11.30 ± 5.43	25.2 ± 12.0
	2	10.55 ± 1.96	32.0 ± 4.0
200	1	30.65 ± 9.54	27.6 ± 8.0
	2	23.53 ± 3.76	28.8 ± 4.0
400	1	31.86 ± 4.30	24.4 ± 8.0
	2	23.89 ± 3.58	32.8 ± 5.2
1,000	2	23.31 ± 7.02	33.6 ± 3.2

In experiment 1 PHI units were measured in the serum of each of the 15 animals in each group. In experiment 2 they were measured in sera pooled from the 15 animals in each group, and each serum pool was assayed nine times.

^{*}Each value represents the mean \pm standard deviation.

[†]Hepatic ascorbic acid was determined in each animal by the method of Roe *et al.*¹⁰

[‡]Blood was withdrawn on the last day of each study by cardiac puncture, and serum PHI was determined according to the method of Dorfman *et al.*¹¹ with the following modifications. Guinea pig serum (25 μl) was added to 1.0 ml of solution containing 2.5 μg of bovine testicular hyaluronidase (Worthington), 0.02 M phosphate buffer, pH 7.0, 0.001 M MgCl_2 , 0.45% NaCl and 0.01% bovine serum albumin. The hyaluronidase-PHI mixture was incubated for 10 min at room temperature. After 10 min, 0.5 ml of the mixture was added to 0.5 ml of 0.3 M phosphate buffer, pH 5.5, containing 0.2 mg of hyaluronic acid. The reaction was maintained at 37°C for 40 min, and then terminated by the addition of 5.0 ml of acid albumin. The resulting turbidity was allowed to develop for 10 min before reading at 600 nm on a Zeiss spectrophotometer.

intake as a factor in increased PHI in scorbutic animals. PHI and hepatic ascorbic acid were assayed in animals receiving 20 $\text{mg kg}^{-1} \text{d}^{-1}$ of ascorbic acid by oral intubation and then fasted for 0, 24, 48, or 72 h. There was little or no change in PHI or ascorbic acid as a result of fasting.

There can be a tenfold increase in serum PHI in certain diseases⁵. Table 1 and Fig. 2 show a modest but significant increase in the PHI levels of scorbutic animals compared with those receiving 20 mg ascorbic acid $\text{kg}^{-1} \text{d}^{-1}$.

These observations confirm the results of Shack *et al.*⁶ who showed an increase of approximately 40% in the PHI of scorbutic guinea pigs compared with animals on a normal diet. Our results do not support the hypothesis of Cameron and Pauling⁷ that ascorbic acid may ameliorate proliferative diseases by increasing biosynthesis of PHI. We did not find any significant change in the PHI of animals receiving 20–1,000 mg ascorbic acid $\text{kg}^{-1} \text{d}^{-1}$.

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matters arising

High affinity uptake of neurotransmitter amino acids

LEVI and Raiteri¹ have concluded that their results on the uptake and release of ³H-GABA by rat brain synaptosomes indicate that homoeexchange is a major mechanism by which radioactive GABA enters synaptosomes when low substrate concentrations are used. They therefore question the effectiveness of high affinity uptake mechanisms in terminating the postsynaptic actions of this and other neurotransmitter amino acids after their release from presynaptic nerve terminals in the central nervous system (CNS)^{2,3}.

I believe that their conclusion is misleading. It is based largely on two findings. First, that unlabelled GABA or glycine in low concentrations stimulate the efflux of ³H-GABA or ³H-glycine from synaptosomes previously labelled with these amino acids. These experiments were conducted, however, with synaptosomes that had been incubated for 10 min in 0.5 μ M labelled amino acid; from our own experience with such preparations we have found that the accumulation of tritiated amino acid is by this time approaching a steady state, in which the rate of influx is approximately equal to the rate of efflux, that is, a homoeexchange situation may exist. Addition of exogenous unlabelled amino acid in these conditions would therefore be expected to lead to the increased efflux of labelled amino acid observed by Levi and Raiteri¹.

These results do not contradict the hypothesis that the initial rate of influx of labelled GABA or glycine into synaptosomes or brain slices incubated *in vitro* represents a net accumulation of amino acid in the intracellular space. Previous observations⁴ by Levi and Raiteri and our own results⁵ have shown the initial rate of influx of labelled GABA into synaptosomes or brain slices is unaffected either by large increases in the size of the intracellular GABA pool induced by previous incubation in a medium containing a high concentration of the amino acid, or by treatment of animals with the GABA-glutamate transaminase inhibitor amino-oxyacetic acid. These findings are hard to reconcile with the notion that the accumulation of labelled

GABA occurs largely by homoeexchange, as if this were so the initial rate of influx of labelled GABA should be increased in such conditions.

Second, Levi and Raiteri measured the net removal of GABA from the incubation medium when synaptosomes were incubated with ³H-GABA at concentrations from 1 to 10 μ M. They show that the net uptake of amino acid is less than that expected from measurements of the removal of radioactivity from the medium, although approximately 40% of the total radioactivity removed could be accounted for by a net accumulation of GABA. These results, however, are in complete disagreement with those of similar experiments from the same laboratory⁴ which showed that net uptake of GABA accounted for more than 95% of the uptake of ³H-GABA in synaptosome preparations from various regions of rat CNS incubated with 20 μ M ³H-GABA. Aprison and McBride also observed a net accumulation of glycine in rat spinal cord synaptosomes when these are incubated with low concentrations of glycine (37.5–150 μ M).

The results of Levi and Raiteri also disagree with our previous findings² which showed that a net uptake of GABA accounted for more than 80% of the total uptake of ³H-GABA in rat brain slices incubated with 200 μ M GABA. In these conditions the uptake of exogenous GABA led to more than a threefold increase in the total GABA content of the tissue.

Nevertheless, after a 30 min incubation with this relatively high concentration of exogenous GABA there was also a significant exchange between the endogenous GABA content of the tissue and ³H-GABA in the external medium². Such exchange is not unexpected and does not contradict the conclusion that high affinity transport mechanisms for amino acids are capable of a net removal of amino acid from the external medium, and can thus represent important mechanisms for terminating the actions of these pharmacologically active substances in the CNS.

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- ¹ Levi, G., and Raiteri, M., *Nature*, **250**, 735 (1974).
- ² Iversen, L. L., and Neal, M. J., *J. Neurochem.*, **15**, 1141 (1968).
- ³ Logan, W. J., and Snyder, S. H., *Brain Res.*, **42**, 413 (1972).
- ⁴ Levi, G., Bertollini, A., Chen, J., and Raiteri, M., *J. Pharmac. exp. Ther.*, **188**, 429 (1974).
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- ⁶ Aprison, M. H., and McBride, W. J., *Life Sci.*, **12**, 449 (1973).

LEVI AND RAITERI REPLY—When synaptosomes are incubated in a definite volume of medium containing a very low ³H-GABA concentration, the steady-state accumulation that is approached after a few minutes does not correspond to a saturation of the synaptosomal uptake capacity, but is a relative value which depends on the concentration of GABA in the medium and on the ratio between the amount of tissue and the volume of medium. If the same amount of synaptosomes is either incubated in a larger volume of medium or, even more so, if it is superfused with this medium, uptake continues to increase for a much longer period. Moreover, similar uptakes of 10 and 20 μ M ³H-GABA were obtained in synaptosomes that had been preincubated in the presence or in the absence of 0.5 μ M unlabelled GABA, and similar results were obtained in superfusion exchange experiments when synaptosomes were preincubated in 0.05 μ M ³H-GABA instead of 0.5 μ M. For these reasons the synaptosomes used in our experiments¹ must be considered as prelabelled, and not as preloaded to an "absolute" steady state.

If one accepts this concept, and assumes, as is generally done, that the labelled GABA mixes with the endogenous pool, then our results do show that homoeexchange is an important mechanism by which labelled GABA is accumulated by synaptosomes, particularly when low GABA concentrations are used. As homoeexchange has never been considered to contribute significantly to the initial rate of uptake of radioactive GABA by nerve terminals, in our opinion it is not possible, at present, to say whether the net uptake of this amino acid (which we did not deny) has or has not a high affinity component (K_m between 4×10^{-7} M (ref. 2) and 2×10^{-5} M (ref. 3)). We suggest that, even if, a high affinity component did not exist, a low affinity uptake system (K_m of the order

of 10^{-3} M) might still be effective for the synaptic inactivation of GABA. The data of Fonnum *et al.*⁴, who estimated the concentration of GABA in 'gabergic' nerve terminals to be "at least 60 mM, probably over 100 mM", seem to support this suggestion.

As to the other comments raised, we are obviously aware of the fact that our present data disagree with previous experiments conducted in our own as well as in other laboratories. This did not seem to us a strong enough reason not to publish the data. Moreover, the disagreement is often more apparent than real, and in most cases concerns the interpretation of the data rather than the data themselves. For example, the finding² that cortex slices (which contain structures other than nerve endings) show a net uptake of GABA after 30 min of incubation in the presence of a concentration (200 μ M) at which high affinity uptake^{3,5,6} and exchange¹ are virtually saturated, does not disprove that homoeexchange can account for a large part of the initial rate of accumulation of radioactivity by synaptosomes incubated with 1 or 10 μ M ³H-GABA.

Similarly, our data on glycine (detectability of homoeexchange in spinal but not in cortical synaptosomes) are not disproven by those of Aprison and McBride⁷ who did show a net uptake of unlabelled glycine, but did not assess the contribution of exchange to the accumulation of radioactive glycine. Moreover, these authors may have somewhat overestimated net uptake, as they did not consider the increase in glycine concentration that might have occurred in the tissue on incubation without added glycine⁸.

The similarity between radiochemical and chemical uptake of 20 μ M GABA that we found in a previous study⁹ is difficult to explain at present. In this type of experiment the choice of a correct type of control is very critical, and we are now trying to determine whether an overestimation of net uptake might have resulted from the type of control chosen.

The results of the experiments in which the tissue concentration of GABA was artificially increased^{9,10} could have several explanations: for example, the increased intracellular concentration of GABA may cause a concomitant inhibition of net uptake and a stimulation of homoeexchange. In appropriate experimental conditions we have shown that the accumulation of radioactive GABA is increased in synaptosomes prepared from amino-oxyacetic acid-treated rats.

We take this opportunity to mention that one of the data reported in Fig. 2 of our letter¹ was wrong, because of a trivial technical error. The figure showed a rapid and massive depletion of synaptosomal GABA content and

the absence of any detectable homoeexchange on superfusion with a sodium-free medium. Our latest data (Raiteri *et al.*, unpublished) indicate that superfusion with sodium-free medium (NaCl replaced by sucrose) does not cause a significant increase in the spontaneous release of ³H-GABA from purified synaptosomes. They do confirm, however, that, in the absence of sodium—homoeexchange is 95–100% inhibited. Therefore, the error does not invalidate the conclusion that absence of homoeexchange could account for what looks like inhibition of the high affinity uptake of GABA.

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- ¹ Levi, G., and Raiteri, M., *Nature*, **250**, 735 (1974).
- ² Henn, F. A., and Hamberger, A., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 2686 (1971).
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- ⁶ Levi, G., and Raiteri, M., *Life Sci.*, **12** (1), 81 (1973).
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- ⁸ Bradford, H. F., and Thomas, A. J., *J. Neurochem.*, **16**, 1495 (1969).
- ⁹ Levi, G., Bertollini, A., Chen, J., and Raiteri, M., *J. Pharmac. exp. Ther.*, **188**, 429 (1974).
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Playing possum

IN his interesting article on the opossum α haemoglobin chain sequence, Stenzel¹ states that 'a selectionist interpretation of the more rapid rate of molecular evolution in a living fossil is possible' because we may expect more rapid molecular evolution 'accompanying slow morphological changes because the reduced number of loci undergoing substitutions may sustain more intense selection per locus'.

Perhaps this is so, but selectionists can cite scripture for their own purpose. Neutralists, however, are delighted to find that this molecule of a living fossil underwent changes at least as rapidly as the

homologous molecules in highly-evolved species. This supports the thesis that 'there seems to be considerable latitude at the molecular level for random genetic changes that have no effect on the fitness of the organism' (ref. 2).

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¹ Stenzel, P., *Nature*, **252**, 62 (1974).

² King, J. L., and Jukes, T. H., *Science*, **164**, 788 (1969).

Sunspot cycle periodicities

THE Maximum Entropy Spectral Analysis technique of Burg (unpublished) and Cohen and Lintz¹ has led to the discovery of periodicities in sunspot data and an ability to predict ionospheric reflectivity and climatic features. But the theory that a planetary influence affects sunspot variations^{2–4} still cannot be ruled out, as is shown by a further analysis of the data of ref. 1.

I accept the 11.05 ± 1.5 , 9.8 ± 0.1 , and 8.15 ± 0.15 yr terms found by Cohen and Lintz but wish to choose another way of describing the cause of their 89.6 yr peak. The proposed fundamental beat tone of 179 yr just does not appear in their periodogram nor is it the beat period suggested. At about one half that value (also the true value of the beat period they have proposed) there is a fairly broad resonance. But 89.6 yr (really more like 86 skewed) is the value one obtains when oppositions and conjunctions of Uranus and Neptune are considered as a possible cause. The relative phase of these two planets is at quadrature during the peak sunspot maxima of 1778, 1864 and 1950 and in conjunction or opposition at the least sunspot maxima of 1821, 1907 and 1993 (predicted). I found the same periodicity but different significant phase relationships when I compared the relative positions of Uranus and Neptune to peak periods of energy released by terrestrial earthquakes². Because of the special phase relationships between the sunspot and planetary data, I feel the 86 yr 'tone' is really due to Uranus and Neptune.

Interestingly, the other periodic terms which Cohen and Lintz found can be explained as the resonance of the following planetary mean motions: (1) $J + S + U - N = 1/8.06$ yr; (2) $J + U + N = 1/9.78$ yr; (3) $J + U - N = 1/11.09$ yr.

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¹ Cohen, T. J., and Lintz, P. R., *Nature*, **250**, 398–399 (1974).

² Bagby, J. P., *The Moon*, **6**, 398–404 (1973).

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⁴ Wood, K. D., *Nature*, **240**, 91–93 (1972).

Matters arising

Matters Arising is meant as a vehicle for comment and discussion about papers that appear in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted. Neither contribution nor reply (if one is necessary) should be longer than 300 words and the briefest of replies, to the effect that a point is taken, should be considered.

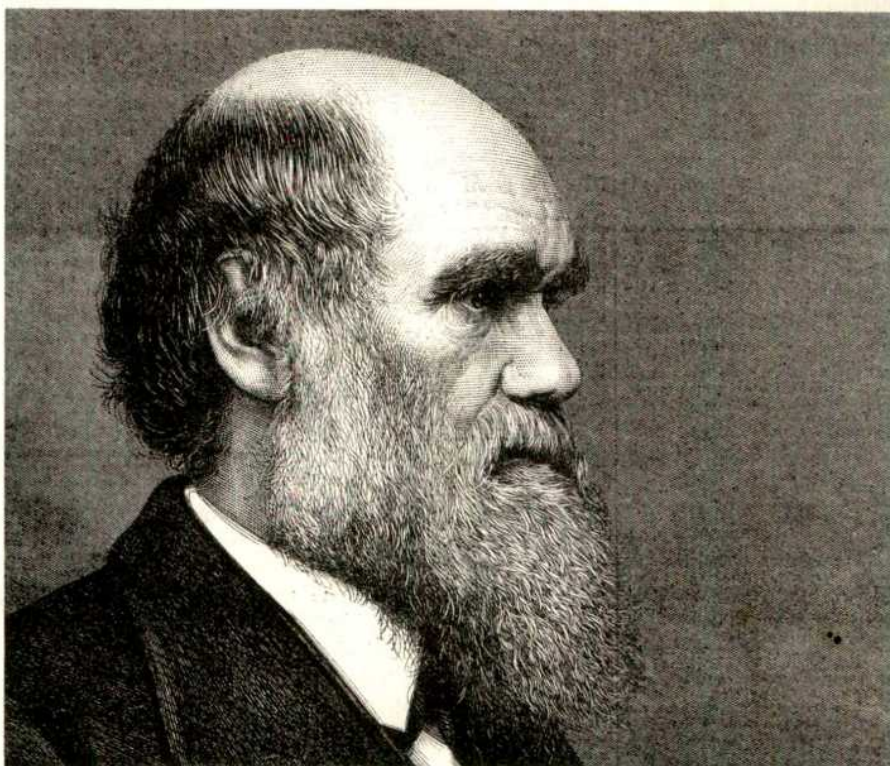
reviews

Missing some of Darwin's brilliance

Darwin on Man: A Psychological Study of Scientific Creativity. By Howard E. Gruber. (Together with Darwin's early and unpublished notebooks, transcribed and annotated by Paul H. Barrett.) Pp. xxv+495. (Wildwood House: London, September 1974.) £5.00.

HOWARD E. GRUBER and Paul H. Barrett have produced a book which they hope will elucidate the roots of Charles Darwin's achievement. They believe that the six notebooks which Darwin kept in the years 1837-39 contain crucial evidence for an analysis of his scientific creativity. Sir Gavin de Beer and his collaborators have already published the four notebooks on the transmutation of species. The Gruber-Barrett volume now presents for the first time a complete transcription of Darwin's two notebooks (designated M and N) on man, mind, and materialism, along with several of his other unpublished contemporary documents on the same subjects. They also reprint some crucial passages from previously published documents, primarily the notebooks on transmutation of species. Barrett transcribed the manuscript materials and has provided detailed bibliographic notes, which are supplemented by Gruber's useful commentaries summarising both the M and N notebooks.

Gruber's primary contribution to the volume is a 257 page analysis of Darwin's scientific creativity, in which the M and N notebooks play a prominent role. Gruber presents two major theses. He first argues that Darwin's invention of the theory of evolution by natural selection between 1837 and 1839 was not the result of a "golden moment of insight", but was instead the culmination of a gradual process involving many levels of thought. This illustrates the belief of Gruber (and Jean Piaget, who wrote a laudatory foreword to the book) that creativity is generally a more gradual process than most people realise. His second thesis is that the M and N notebooks hold "the key to understanding the essential role of his ideas about man and mind in his thinking about evolution". Gruber concludes



Charles Darwin: spent his life refining the original theory

that Darwin's materialism is evident in these notebooks, and that he delayed publication of his conception of evolution by natural selection because he feared persecution and ridicule for his materialism.

Gruber's thesis that Darwin's creation of the theory of evolution by natural selection was a gradual and cumulative process is convincing. But scientific creativity is elusive, and the details of Gruber's analysis are fraught with difficulties. His chapters on the Darwin family *Weltanschauung* and Darwin's teachers contain precious little evidence about actual influences upon Darwin himself. The crucial evidence about his scientific creativity comes from the notebooks. Yet what are these notebooks? They contain Darwin's hurriedly written impressionistic speculations, including his gleanings from other literature mixed indistinguishably with his own ideas. Many of the passages, especially in the M and N notebooks, are unclear or contradictory. We have no way of knowing which passages Darwin considered important or trivial at the time that he wrote them. We can, however, agree with Darwin's later assessment that much in the notebooks is trivial or worthless, even for the historian. To

recreate Darwin's creativity from these notebooks is terribly difficult.

To simplify matters, Gruber explicitly ignores Darwin's ideas about heredity, a serious omission which he acknowledges. He then proceeds, like a classical scholar working on Anaximander's one fragment, to make much of little. He finds in Darwin's notebooks an "underlying order" which completely escapes me. In constructing logical systems out of Darwin's speculations, he is led into contradictions. For example, Gruber argues that Darwin's brief mention of "monads" is really a whole theory of evolution, and that the "monad theory contains the principle of spontaneous generation". Later, Gruber argues that Darwin gave up the theory of spontaneous generation, influenced by Ehrenberg's claim that extant lower organisms were identical with fossil forms. The problem with this scenario is that Ehrenberg is mentioned in the very first passage of Darwin's supposed 'monad theory'. So although Gruber's thesis about the gradual nature of Darwin's creativity is reasonable, the details of his creative process remain obscure.

Gruber's second thesis, that the M and N notebooks and associated docu-

ments contain much about Darwin's views on man, mind, and materialism in relation to evolution, is certainly true. Indeed, the major significance of this book lies in showing that at a very early stage Darwin understood that his idea of evolution by natural selection had vast materialist implications for human evolution and psychology.

Yet Gruber's conclusion that Darwin put off publication of his ideas primarily from fear of persecution and ridicule for his materialist ideas is drawn from shaky evidence. Gruber even resorts to interpreting a dream briefly reported by Darwin, and argues that a photograph of Darwin taken in 1854 reveals "the strain of long years of delay".

A different interpretation is possible. Darwin had high standards for his published work. He later commented that he benefitted from the delay in publishing his works on evolution because he was able to gather better evidence and think through possible objections. Perhaps he was motivated not so much by fear of ridicule as a materialist, as by the feeling that his own standards of argument and evidence had not been attained.

After giving the impression that he admires Darwin's materialism, Gruber's last chapter, "Creative Thought: The Work of Purposeful Beings", contains a surprise. His theory of creativity is vitalistic nonsense. It follows the tradition of Alfred Russel Wallace's mysticism (after 1864) rather than Darwin's materialism. Gruber's theory is that each individual organism functions "according to its own internal laws or organisation". When two organisms interact, the result is

behaviour which is utterly unpredictable from a consideration of the special laws governing the individual organisms. A human has, in addition, an internal unpredictable creativity resulting from the interaction of the separate parts of his thought, each of which has a life of its own. So in the end the reader discovers that in his analysis of Darwin's creativity, Gruber is conducting the inquiry under the assumption that the creative process is inherently inscrutable. Darwin spent his life reducing complex biological behaviour to materialist laws. He intensely disliked Wallace's mysticism about human minds, and he would have been appalled to have Gruber's vitalistic theory applied to himself.

Does this book as a whole open up a fruitful field of Darwin research? I hope not. By focusing almost entirely upon Darwin's theory construction of 1837-39, the authors have missed the most important and influential part of Darwin's genius. As he himself believed, Darwin is important not because he outlined the theory of evolution by natural selection in the late 1830s, but because for the rest of his life he refined and supplemented the theory, found evidence for it, and presented it in enormously influential published works. Michael Ghiselin's *The Triumph of the Darwinian Method* (The University of California Press: Berkeley and London, 1973) is a step in the right direction, but the vast and important problem of Darwin's influence through his letters and published works in the second half of the nineteenth century has scarcely been touched. The M and N notebooks seem inconsequential in comparison. **William B. Provine**

Elementary world climatology

World Climatology. By John G. Lockwood. Pp. xiv+330. (Arnold: London, March 1974.) £8.50.

THE study of climatic change and its effect on the environment is one of the most important branches of modern science, in terms of the potential direct effects on the lives of non-scientists. As a result of developments in several fields including studies of the Earth's magnetic field and astronomy, as well as the more conventional aspects of meteorology (which now include use of satellite observations and high speed computers), a synthesis leading to an understanding of at least some aspects of climatic change seems to be in the offing. As a result, many people who have no formal training in meteorology are working in areas where some detailed understanding of the working of the atmosphere is essential. Those people would find this book invaluable.

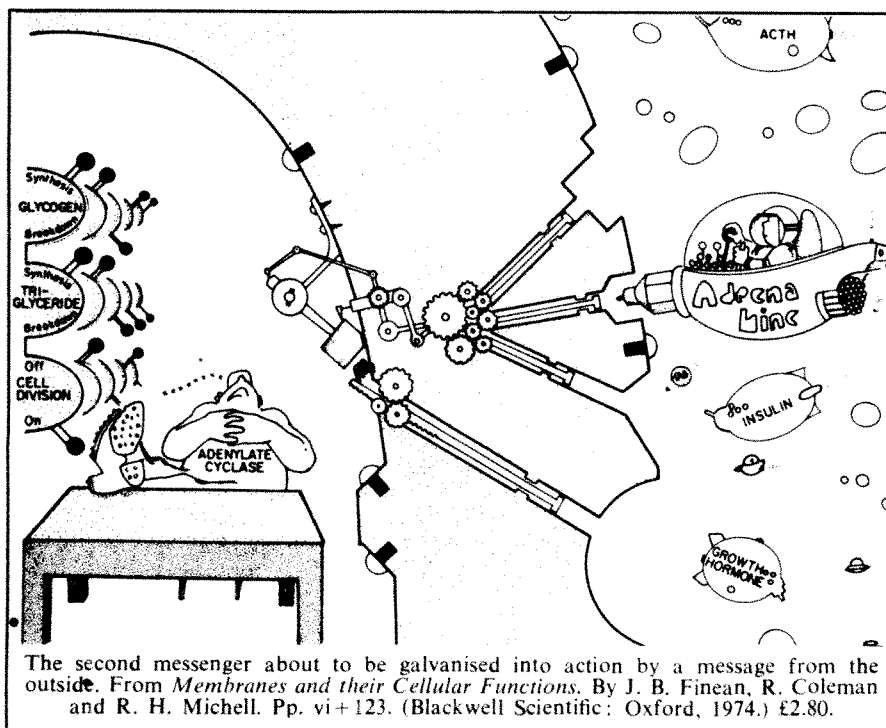
World Climatology is not concerned with climatic change at all, but with the physical basis of climatology with particular reference to environmentally important aspects. It is intended, says the author "for students taking university courses on climatology, particularly those at an advanced level" and the reader is warned that an acquaintance with basic concepts of meteorology, mathematics and physics is required. But these assumed basics are, in fact, at the most elementary level, and the book introduces gently such concepts as the Stefan-Boltzmann law of black body radiation.

The first part of the book deals with the basics of climatology: radiation laws, convection and turbulence, precipitation and evaporation, and local microclimates (ranging down to that at the surface of a plant leaf). The second and third parts apply these basics to the low latitude atmosphere and to the atmosphere of middle and high latitude regions. With a generous sprinkling of figures—one or more clear and informative line drawings on most pages—and well written text this makes the book ideally suited for its intended readership, but equally valuable to anyone trying to fill in a knowledge of climatology which is sketchier than it should be.

The publishers of this book have done an excellent job. The clarity of the figures is matched by the clarity of the text. There is a generous bibliography, in the form of references at the end of each chapter.

This book is a good place to find out about the basics of climatology.

John Gribbin



The second messenger about to be galvanised into action by a message from the outside. From *Membranes and their Cellular Functions*. By J. B. Finean, R. Coleman and R. H. Michell. Pp. vi+123. (Blackwell Scientific: Oxford, 1974.) £2.80.

The Physics of Time Asymmetry. By P. C. W. Davies. Pp. xviii+214. (Surrey University Press; Leighton Buzzard, 1974.) £6.50.

THIS book is about what is popularly called the 'arrow of time' but the author does not much like the term because the asymmetry is that of the world in respect to time, not of time itself. The book is a critical study of the origin of the asymmetry and of its role in basic physics. Indeed, it could serve as the making of an excellent course on the foundations of physics.

With the only known exception of properties of K mesons, the laws of physics are symmetric in regard to time, and so the asymmetry must arise from boundary conditions. Davies proceeds from that consideration to two important inferences. First, that irreversibility in the Universe depends ultimately on gravitation, since any sizeable body in the Universe is held together by its own gravitation and there is no permanent equilibrium state for such a body. Second, that all important aspects of time asymmetry are traceable to the beginning or end of the Universe. Both bring the subject naturally into the realm of cosmology. Appropriately, the chapter in the centre of the book is on "Thermodynamics and cosmology". Moreover, the fact that in ordinary electromagnetism we consider only retarded effects is nowadays generally explained by the 'absorber' theory of radiation, and so it again relates the subject to cosmology. Whether or not the thermodynamic and electromagnetic properties are independent seems to remain an open question. Davies discusses all that in his next chapter. Thermodynamic irreversibility is bound up with probability theory, and a more fundamental sort of probability is involved in quantum mechanics, as is then described. Finally, Davies discusses the bearing of the whole subject upon the choice of a cosmological model.

Davies skilfully conveys the flavour of the mathematical treatment without going into its details. His presentation tends to be over-modest, and this inhibits him somewhat from stating categorically the conclusions reached at each stage of the development of the topic—the reader has to do a little searching to find them. Nevertheless, "Having completed this book", he makes bold to conclude that (Introduction, page 7), "the basic framework for understanding the nature of time asymmetry is clearly available, and it is unlikely that any future considerations of detail will introduce anything qualitatively new". Although the book certainly seems to do more than any

other towards achieving this happy outcome, one is left with an uneasy feeling that something is still missing. The old problem of the relationship between the indeterministic world of quantum physics and the fully deterministic world of general relativity seems still imperfectly understood. Actually, Davies exposes the difficulties in his section 6.3, "Quantum measurement theory". I cannot but conjecture that the place of the observer will somehow have to be recognised more extensively in formulating physical theory and that this will entail the admission of uncertainties associated with other physical constants besides Planck's constant.

The book most adroitly brings together all the main relevant lines of thought; whatever remains to be done must surely start from the ground so well surveyed by Dr Davies.

W. H. McCrea

Time and space

Space, Time and Spacetime. By Lawrence Sklar. Pp. xii+423. (University of California Press: Berkeley, Los Angeles and London, 1974.) \$15.00; £7.50.

LAWRENCE Sklar is a philosopher writing about philosophical aspects of space and time, from Newton and Leibniz to Einstein and Reichenbach. Nevertheless, his clear grasp of modern mathematics and physics, and his lucid exposition, make this book eminently suitable for physicists and mathematicians who wish to explore the philosophical background to their subject. Although the text is not in any sense mathematical, crucial topological and geometrical concepts are carefully explained before they are incorporated into the author's thesis.

The book is divided into four main sections. The first deals with the epistemology of geometry with a historical development leading up to Minkowskian and Riemannian spacetimes. Much attention is then devoted to Poincaré's proposition that the geometrical structure of the world is merely a matter of convention and to the existence of different hypotheses each equally capable of explaining the same observations. The complex web of argument and counter argument subdivides into many alternative points of view and may well frustrate the scientific reader who probably regards the whole issue as sterile overemphasis, especially as the author terminates the controversy with an admission of inconclusiveness.

Part two is concerned with absolute motion and the theory of relativity, tracing the controversy between the substantial view of Newton that space is an entity and the relational views of Leibniz and Mach that it's not there at all. The special and general theories of relativity are somewhat grafted on to this discussion without, of course, solving the problem. Sklar gives a fine descriptive account of the theories of relativity, which could well be used by physics students wishing simply to learn the subject. Even modern approaches such as geometrodynamics get a mention. At the end of this section the author presents his own rather unconvincing answer to the problem of the origin of inertial forces. Rejecting Mach for apparently aesthetic reasons and wishing to retain a pure relationist stance, he proposes that the statement "is absolutely accelerated" is not a relational term, giving it instead the status of a complete assertion. This may well be a valid philosophical contortion, but it is unlikely that the physicist will be satisfied with the denial of any explanation for inertial forces.

Part three of the book deals mainly with time—the usual problems of simultaneity, time dilation, twins paradox and so on in relativity theory—and also presents some bold treatment of bizarre topologies, including a discussion of trouser spaces, non-orientable manifolds and closed time-like world lines. The discussion is framed in the causal theory of time to which the last part of this section turns, concluding with the widely held belief that the theory is implausible.

The final section summarises briefly the fascinating and controversial subject of time asymmetry. The account suffers somewhat from superficiality, and the vital topic of Reichenbach's branch systems is not discussed in detail. The conclusion given to this section is that we simply 'know' the relation of temporal priority between events. This is a most unsatisfactory escape route, which does nothing to dispel the lamentable confusion which exists between the phenomenon of the psychological flow of time and time asymmetry as encountered in the objective world, and which is so prevalent among scientists and philosophers who write about time asymmetry.

This book is a comprehensive, well written and authoritative account of space and time which well suits both scientists and philosophers of science. It is inclined to be a little tedious and repetitive, but contains plenty of exciting and interesting material for students of these disciplines. P. C. W. Davies

obituary

Herbert Alexander Sober, a major innovator and developer of our current technology for the separation and purification of biologically important macromolecules, died of a heart attack in Washington, D.C., on November 26, 1974, following a relatively minor operation.

Sober was an undergraduate at the City College of the City of New York and received his Ph.D. degree in 1942, majoring in biochemistry at the University of Wisconsin. Following his graduate studies, he spent three years as a toxicologist and as Chief of the Analytical Research Group at Edgewood Arsenal near Baltimore. After two years at Mount Sinai Hospital in New York he joined the National Cancer Institute, NIH, as a member of the Commissioned Corps of the US Public Health Service, serving as Chief of the Laboratory of Biochemistry. In 1968 he transferred to the US Civil Service and took the position of Chief in the Laboratory of

Nutrition and Endocrinology, National Institutes of Arthritis, Metabolism, and Digestive Diseases. Sober is best known as co-developer, with Elbert Peterson, of the modified cellulose ion exchangers. These substances, which could be prepared in a wide variety of forms having either cationic or anionic characteristics, and possessing a wide range of intrinsic pK values, are extremely gentle to sensitive protein and nucleic acid systems and also exhibit excellent discrimination when combined with the principle of gradient elution for the separation of macromolecular mixtures. There are very few papers published in the biochemical or molecular biological literature that do not quote the use of the cellulose derivations as key tools in the experiments described. The value of these chromatographic techniques was recognised, in 1970, by the selection of Sober and Peterson for the Hillebrand Award, presented by the American Chemical Society of Washington.

The association of Sober's name with chromatography and its applications has tended to obscure his contributions to a number of other areas. He published nearly 150 papers during his career, including the ever-useful *Handbook of Biochemistry*. He was, for example, involved in the first isolation of pure crystalline pyridoxamine and pyridoxal phosphates. In the last few years he and his colleagues, particularly Dr Robert Simpson and G. W. Rushizky, made major advances in our understanding of the interaction between basic oligopeptides and polynucleotides, particularly when applied to research on the chemical nature of chromatin.

Herbert Sober was, above all, a man of great balance and human warmth. He was universally respected by his coworkers and students over the years and maintained, throughout his professional life, a blend of vigorous interest in science and a rare ability to share his love, wisdom, and simplicity with family and friends alike.

announcements

Appointment

Alasdair D. Berrie, Officer-in-charge of the River Laboratory of the Freshwater Biological Association at East Stoke, Wareham, Dorset, has been accorded the title of Visiting Professor in the University of Reading.

Miscellaneous

Biogeography Study Group. At the annual conference of the Institute of British Geographers, this Group was formally constituted. It aims to foster the study and development of biogeography by discussion and field meetings, and by providing contact with those people working in related disciplines. A symposium is scheduled for September, 1975, to be held on the North York Moors. Further information may be obtained from: Dr L. F. Curtis, Department of Geography, University of Bristol, Bristol, UK; Mr J. A. Taylor, Department of Geography, University College of Wales, Aberystwyth Dyfed SY23 3DB, Wales; Secretary, Institute of British Geographers, 1 Kensington Gore, London SW7 2AR, UK.

Fractionation Services. The American Red Cross National Fractionation Center, supported by the National Heart and Lung Institute, is offering facilities to an investigator, for the pilot-plant or large-scale production of blood proteins, enzymes, membrane fractions and so on. Further information from: Dr M. Wickerhauser, Director, American Red Cross National Fractionation Center, 9312 Old Georgetown Road, Bethesda, Maryland 20014.

METASERV prize. The Institute of Metallurgists has organised a competition in Metallurgy with prize money totalling £200 (including a special student Prize). The closing date for the competition is July 31, 1975. Further details may be obtained from: *The Metallurgist* or The Institution of Metallurgists, Northway House, High Road, Whetstone, London N20 9LW, UK.

International meeting

April 27-May 3, **Stereochemistry**, Lucerne, Switzerland (Professor J. Dale, Department of Chemistry, University of Oslo, Blindern, Oslo, Norway).

Reports and publications

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UMIST 1824-1974. Pp. 104. (Advance No. 15.) (Manchester: University of Manchester Institute of Science and Technology, 1974.) [512]
Scholarships Guide for Commonwealth Postgraduate Students, 1975/1977. Pp. 310. (London: The Association of Commonwealth Universities, 36 Gordon Square, WC1, 1974.) £2.50. [612]
The Zoological Record, 1970, Vol. 107, Section 1: Comprehensive Zoology. Compiled by J. Fitzgibbon. Pp. vi + 68. £3.65. 1970, Volume 107, Section 3: Porifera together with Archeocyatha. Compiled by S. Ware. Pp. v + 16. £3.65. 1970, Volume 107, Section 5: Echinodermata. Compiled by A. M. Clark. Pp. v + 51. £5.15. (London: The Zoological Society of London, 1974.) [612]
Agricultural Research Council; and Medical Research Council. Food and Nutrition Research: Report of the ARC/MRC Committee. Pp. xv + 211. (London: HMSO; Amsterdam and New York: Elsevier Scientific Publishing Company, 1974.) £3.80 net. [912]
The Responsibility of the Governors. By Sir Michael Swann, FRS. (BBC Lunch-Time Lectures, Ninth Series, 1.) Pp. 14. (London: BBC, 1974.) [912]
A Second Appendix to the Second Edition of an Index of Mineral Species and Varieties Arranged Chemically. By Max H. Hey and Peter G. Embrey. Pp. xii + 168. (London: British Museum, (Natural History), 1974.) [912]

Other countries

Bibliography of Laser Doppler Anemometry Literature. Edited by F. Durst and M. Zare. Pp. 55. (Copenhagen: DISA Elektronik; Bristol, DISA UK, Techno House, Redcliffe Way, 1974.) gratis. [911]
Australia: Commonwealth Scientific and Industrial Research Organization. Division of Soils Technical Paper No. 23: Laboratory Procedures for Cation Exchange Measurements in Soils. By B. M. Tucker. Pp. 46. (Melbourne: CSIRO, 1974.) [911]
National Research Council Canada. NRCC No. 13686: A Method of Monitoring Nationally for Possible Delayed Effects of Various Occupational Environments. By H. B. Newcombe. (Associate Committee on Scientific Criteria for Environmental Quality.) Pp. 42. (Ottawa: National Research Council, 1974.) [911]

nature*February 13, 1975*

No longer so much solid ground

A BETTER public understanding of science is an objective being pursued in many parts of the world at present. Committees are constituted to look at the interfaces between science, politicians and the media. Interviewers are despatched to ask the man-in-the-street whether he knows what electricity is, and if so, who told him. Sociologists are busy clipping newspapers and measuring science coverage to the nearest centimeter. And science journalists are being called on to explain why the popular press will run stories on cancer cures in preference to those on radiotelescopes. The driving force for all this activity is undoubtedly a widespread belief that the public is disenchanted with science, and the corollary that an improvement in the quality or quantity of public exposure might result in a better appreciation of the virtues of the pursuit of science.

The very existence of a broadly based public disenchantment must be questioned, the more so since everyone seems to believe in it these days. Ask someone at random what he doesn't like about science and he will probably say "the atomic bomb", or "pollution", or "spending so much money on going to the Moon". After a period of idolisation, scientists have to come to terms with the fact that, like politicians, economists, lawyers and industrialists, they now have to live in a world in which they face frequent criticism about events far too complex to have an identifiable source. This is hardly disenchantment, more an entry into a world in which cynicism is more common than trust, and it is difficult to see that any public relations campaign will succeed, or even that it should be attempted.

There are, however, two kinds of people whose perception of science we ought to worry about: the politician, because he may want to use our results and because he holds the purse strings, and the young student, because he has it in his power to provide or withhold the manpower. To the extent that any politician cares about science at present, a common complaint one hears is that scientists don't seem capable of proffering clear-cut advice. The schoolboy, more forthcoming, may say that science is too complex and offers no obviously profitable pastures these days. Here there is scope for a careful

examination of how the scientist is depicted.

The problem is that, in many minds, a nineteenth century image of the scientist still persists—there are even those within the community who lack sufficient curiosity about their profession to have perceived that the scientist can no longer be portrayed as a bearer of truth. This is not to say that scientists are bearers of lies these days, but it is to assert that the character of most science is now so complex, particularly when dealing with man's interactions with the world, and the purposes for which research is pursued are so varied, that any generalisations that a scientist makes in the literature (and few scientists are happy to let someone else do the generalising for them) are written not on tables of stone, but in chalk, with a good chance of being erased within the year. Scientific statements are falsifiable statements (even though few scientists have read Popper) and in their very nature many of them are indeed going to be falsified.

'Maker of falsifiable statements'—this is hardly a generally understood view of the scientist, and certainly not the kind of know-all image that scientists are often portrayed in the media as possessing.

Yet without stripping away much of the popular mythology surrounding the scientist and his conduct of science, confusion is bound to continue among those who have been led to believe that the scientist's generalisation, having got past a referee or two, is therefore worthy of exceptional respect.

Outsiders and prospective insiders often learn about the true nature of science (which, it must be said, is much more stimulating and exciting than it would be if scientists' pronouncements were only verifiable) by shrewd observation. But it would be better if they could acquire this information more directly. Any project to increase public understanding of science could well devote much of its energy, not to passing round more facts, but to building up, probably through case histories, an appropriate primer on the way in which science knows in part at any particular time—and doesn't always proceed in the most logical way to build on partial knowledge.

Turkey's flower powers

PETER COLLINS REPORTS

THE recent decision of the Turkish government to make trade in opium poppy "straw" a state monopoly is a logical step in the process of assuming complete control of the production and marketing of this crop. It should also reassure those interested people outside Turkey who were surprised, if not distressed, at the announcement some months ago that the ban on growing the opium poppy (*Papaver somniferum*) had been rescinded. In fact, the lifting of the ban, although said originally to have been suggested for political reasons, was almost essential if widespread distress, possibly starvation, were not to be inflicted on the many thousands of farm families for whom this crop provides almost the sole source of income.

The story of this latest phase in poppy cultivation in Turkey goes back to the beginning of the 1970s. At that time, the United States government, acutely aware of the threat of heroin addiction among its younger population, came to regard Turkey as the main source of the opium from which that drug is derived. Thanks largely to the work of the United Nations Narcotics Laboratory in Geneva, it is possible to establish the provenance of opium samples with reasonable accuracy but in any event there is ample other evidence to support this view, although only a small proportion of the world's total opium is grown in Turkey. Determined to deal with the heroin problem, the United States persuaded the Turkish government to prohibit all cultivation of the opium poppy, an announcement made somewhat dramatically at a meeting of the United Nations Commission on Narcotic Drugs in Geneva in 1972. Moreover, it is certainly true that, following the ban, there was a rapid decline in the amount of heroin coming on the market, not only in the United States but also in the Riviera, where there was at one time said to be a "heroin famine".

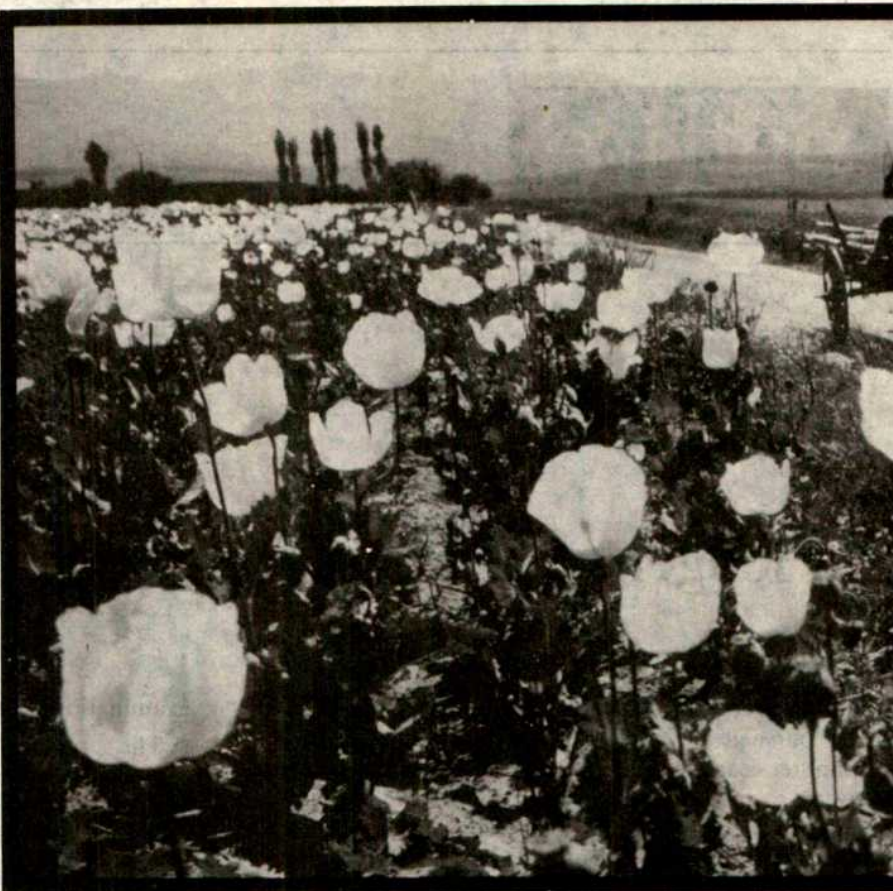
The Turkish ban was imposed on the understanding that the United States would provide the funds—estimated at \$35 million—for recompensing the farmers who were being deprived of their main cash crop, and for the very large research and development programme required to find alternative

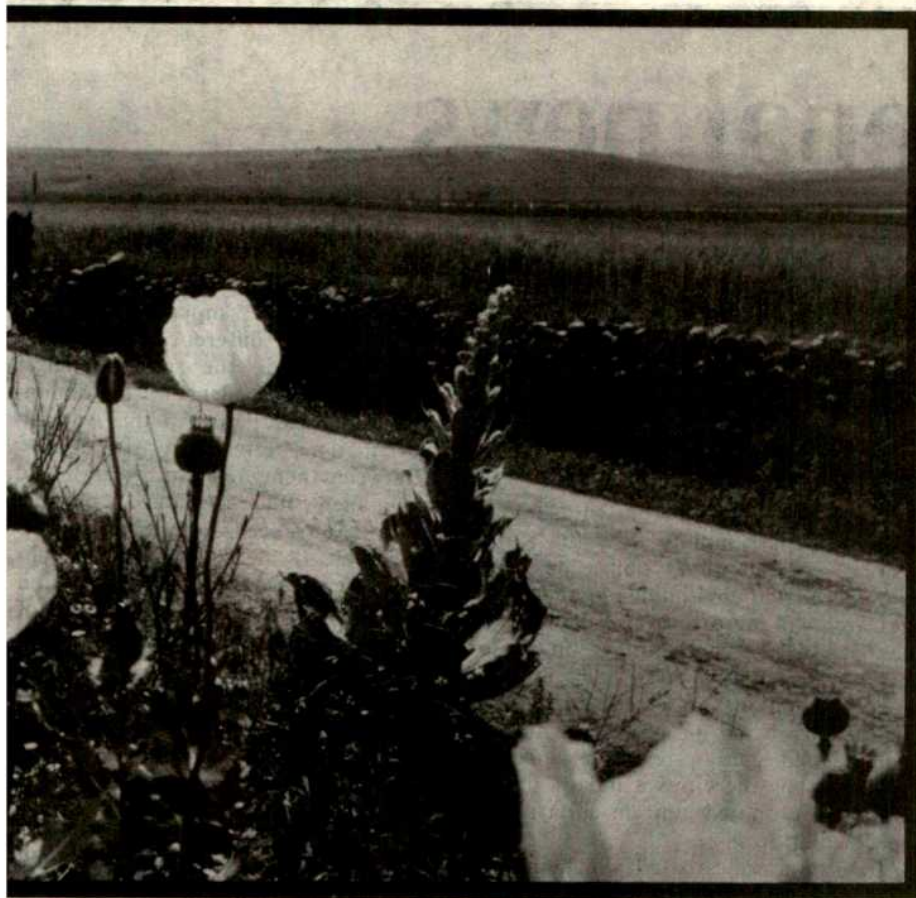
crops or other occupations. In the event, while several million dollars were handed out as compensation, the crop-substitution programme never really got off the ground—a factor that may also have influenced the Turks in their change of mind.

The fact is that in those areas of Turkey where the poppy has traditionally been grown, almost no other cash crop will succeed. Poor soils, low rainfall, extremes of heat and cold make normal agriculture difficult, often impossible, over much of those provinces—Afyon, Burdur, Denizli, Isparta—in the south-western part of the Anatolian plateau. To the problems posed by soil and climate were added that natural resistance to change of any agricultural population, especially when that change was apparently being made to please a foreign power for whom in any event they had no particular affection. In fact, the difficulty of substituting new crops, and ideas amounting almost to a new way of life, for opium poppy cultivation has been known ever since the late 1950s when the UN Food and Agriculture Organisation (FAO) became involved in this type of operation in Iran; and the Americans, with their teams of willing but perhaps oversophisticated experts under AID, were even less successful than previous efforts in this direction. It seems almost as though AID was landed with a vast project which it would almost certainly

have rejected had it been suggested to it in the ordinary way of technical assistance.

At this early stage, the United Nations Division of Narcotic Drugs (the secretariat for the Narcotics Commission) was not involved, and indeed by the summer of 1973 it still had little idea of how the American crop-substitution project was progressing, although there were suspicions that things were not moving very rapidly. What seems to have happened next is that the then opposition party in Turkey held out the removal of a ban on poppy cultivation as part of its election programme. Rather unexpectedly, it found itself in power, and committed to implement its promise. Having effectively snubbed the Americans, the party went to the United Nations for advice. With the arrival of a UN mission in the poppy-growing areas, an unappreciated factor became apparent. It was already known that the situation in Turkey was very different from that in Iran, the other Near Eastern country with a major opium poppy problem. In Iran, opium has for centuries been smoked in the villages in the areas where it is grown. In Turkey the villagers do not smoke opium, but rather the importance of the poppy crop lies primarily in the seed, as the principle source of vegetable oil for the local population and, after extraction, of oil-cake for cattle feed. The rest of the plant is





A poppy field in Turkey: Daily Telegraph picture.

normally used for bedding for livestock. This is not to say that no opium was produced, but the poppy heads were lanced and the opium thus obtained sold, principally when a farmer needed extra funds at short notice, as for example for a wedding or a funeral.

The facts on which this UN 'discovery' were based had certainly been available to the Americans, since it was in examining their very full statistics that the mission was able to confirm what conversation with farmers and other local people had indicated: that there was little growing of the poppy solely as a source of opium. But the Americans had been so obsessed with the idea that 'Turkish opium becomes American heroin' that they had missed the real facts, and gone bull-headed for the complete and immediate prohibition of what was in fact the most important crop for perhaps 100,000 Turkish farmers. Faced with this situation, the Turkish government were to receive some \$35 million for the re-development of the areas affected.

The fact that opium production is not the main objective of Turkish poppy culture added a sound agro- and socio-economic argument to the government's decision to rescind the ban. What will happen now is that the poppy will continue to be grown as before, until the vital two weeks during which the capsules are ripening. It is at this time that, when opium is being produced, the

heads are lanced so that the opium latex can ooze out. It is a laborious business, because the grower must wait 10 to 12 hours before the hardened latex can be collected. Besides keeping several people busy for some hours, this is obviously a difficult process to conceal. It is at this period that an army of government inspectors will descend on the farmers to ensure that no lancing of capsules or illicit sale of materials takes place. Once the capsules have dried, the farmers will be able to collect the seed, both the yield and quality of which is improved when no lancing is carried out. The empty capsules and the upper parts of the stem—the 'poppy straw' of commerce—will later be collected by the government for processing to extract the morphine and codeine for the pharmaceutical industry. In this way opium and hence, heroin, is bypassed completely, yet the farmers will still have the rest of the plant for livestock bedding. Moreover, by setting up their own processing plant, the Turks will not only further reduce any chance of their raw materials getting into the illicit market, but will also acquire a lucrative state monopoly. A second UN mission has in fact only recently returned to Geneva after examining the financial and technological aspects of this further project.

On the ground, plans for putting the government's scheme into action have

been well advanced for some months. Farmers wishing to grow poppies must apply through defined channels, and their applications, with the area it is intended to cultivate, must be duly registered; the total area under this crop has been put at 20,000 hectares (about 45,000 acres) spread over the seven provinces in which it has always been grown. The fact that these poor soils give low yields is of little immediate interest to the government; what matters is that the livelihood of the farmers is assured at least at the previous levels, particularly if the Americans can be persuaded to release the remainder of the \$35 million to help stabilise the situation in the next few years.

It is realised, of course, that this system is unlikely to be completely watertight during the first season, although the UN in Geneva are quietly confident that the Turks will attain their general objective, namely to keep Turkish-grown opium off the world market. The new system will be far simpler and probably cheaper to run than the ceaseless pursuit of illicit opium traffickers, although obviously vigilance will not be relaxed there either. But the government announcement of January 17 makes it quiet clear that the severest measures will be taken against offenders—their harvests will be destroyed, their licences will be cancelled, and no further licence will be granted in the future. Offenders will also be subject to 'the relevant provisions of Turkish penal law'.

A system such as this, especially if it is rigorously enforced, should provide the necessary safeguards to reassure the Americans. Recent reactions from the United States show, however, that they remain unconvinced. One Senator has been quoted by the BBC as saying that it was 'obvious we will be inundated with heroin' now that the ban on opium poppy cultivation is lifted. This alarmist attitude, as reported by the BBC, betrays a complete lack of faith in the power of the Turkish government to control the situation. It may be that, nettled by the failure of their own huge project, the Americans do not credit the facts about Turkish poppy cultivation reported by the two UN missions. But if the Turkish control system goes as planned, no opium at all will be produced and once the proposed processing plant is in operation, in two or three years time, only drugs such as morphine and codeine will come from Turkey.

Whether American pessimism or Turkish confidence will be justified is perhaps anybody's guess, and will remain so until the critical weeks of the poppy seed—or opium—harvest in May this year. □

international news

SINCE energy research and development is a politically popular topic these days, the Ford Administration, which has been searching desperately for something politically popular to announce, has been making much of the fact that massive increases in federal funding for energy technologies were included in last week's budget. The amount has been variously reported as \$1,663 million, \$1,837 million, \$2,115 million, \$2,360 million and \$2,829 million—an array of figures which should further enrich the confusion over energy policy in the United States.

Energy research and development, it should be understood, can be defined in various ways, and various budget figures have been based on different definitions, hence part of the confusion. Another complication is the fact that budget figures are expressed in two different ways—obligations, which represent commitments to pay for grants or contracts although the actual expenditure may not take place immediately, and outlays, which represent the actual amounts of money which each agency will spend in a given year. As far as energy research and development is concerned, there is a considerable difference between total obligations and outlays, all of which makes it a bit difficult to compare what the Ford Administration is proposing to spend next year with what will be spent this year.

Nevertheless, the Office of Management and Budget (OMB), which produced President Ford's budget request, has made an attempt. It has come up with an analysis suggesting that, if Congress agrees to the proposals, obligations for energy research and development will increase from \$1,669 million this year to \$1,837 million next, whereas

Analysing the US energy budget proposals

by Colin Norman, Washington

outlays will increase from \$1,222 million to \$1,663 million over the same period. In addition, obligations for closely related environmental and basic research will increase from \$497 million to \$523 million and outlays on those activities will grow from \$367 million to \$452 million. The total growth in obligations being proposed for direct and related energy research and development is therefore a modest 10%, while the proposed jump in outlays is a spectacular 36%.

It is impossible to tell which individual programmes are being given priority from those figures, however. For that, it is best to turn to a different set of figures—the budget request for the Energy Research and Development Administration, as outlined by Dr Robert Seamans, ERDA's Administrator, to the House Committee on Science and Technology last week. Since ERDA is now responsible for the bulk of the Federal government's energy research and development, the ups and downs of the budgetary fortunes of its various divisions should provide a measure of which energy programmes are being favoured.

Seamans presented the committee last week with a table (see below) which provides details of the agency's budget request for next year. The table is expressed in terms of outlays, and although the total is greater than the

amount shown in the OMB's analysis for the entire federal energy research and development budget (the difference, an ERDA official supposed, probably arises because different things have been included in the summations) it provides further evidence of the dominance of nuclear power in the Administration's long term energy strategy.

Between them, reactor research and development, nuclear materials production and advanced isotope separation technologies—chiefly centrifuge enrichment—are set to receive \$1,118 million next year, which is an increase of \$236 million. Moreover, more than \$400 million is budgeted for capital expenditure on fission technologies, so the nuclear energy programme is again set to carry off more than half of the total energy research and development budget. The fact is unlikely to be accepted by Congress without demur, since there is a groundswell of opinion building up on Capitol Hill that non-nuclear technologies have been relatively neglected.

Although the figures proposed for such items as solar energy, geothermal power, and fusion look generous enough, the actual amounts indicated in ERDA's budget request are unlikely to satisfy most supporters of those programmes. The solar energy budget, for example, which is set to increase in ERDA from \$8.8 million this year to more than \$57 million next, should be seen in relation to Congressional appropriations for 1975 of \$50 million. Furthermore, a panel of experts told the Atomic Energy Commission two years ago that a minimum acceptable programme for solar energy development would involve expenditures of at least \$65 million in the 1976 fiscal year. Similarly, Congress authorised expenditures on geothermal energy in 1975 amounting to some \$44 million, yet the expenditure being proposed for ERDA in 1976 amounts to only just over half that. Although the National Science Foundation still has some programmes involving basic research related to solar and geothermal energy, Congress is unlikely to be satisfied with the amounts being proposed for next year in those areas.

It should also be noted that President Ford has formally proposed to reduce the amounts appropriated by Congress for ERDA in 1975 by some \$85 million; that proposal is reflected in the figures present in his budget. If Congress refuses to go along with that request,

ENERGY DEVELOPMENT PROGRAMMES	1975*	1976*	Change	
		\$ million		
Fossil energy development	195.0	311.3	116.3	59.6
Fusion power research and development	85.0	120.0	35.0	41.2
Fission power research and development	384.1	443.7	59.6	15.5
Nuclear materials production (less weapons activities)	445.2	596.1	150.9	33.9
Advanced isotope separation technology and laser fusion	53.1	78.2	25.1	47.3
Solar, geothermal and advanced energy systems development	34.9	108.6	73.7	211.2
Conservation research and development	16.7	32.2	15.5	92.8
Physical research	281.6	312.5	30.9	11.0
• Environmental and safety research	165.0	197.7	32.7	19.8
Plant and capital equipment for above	553.4	629.2	75.8	13.7
	2,214.0	2,829.5	615.5	27.8

*Fiscal years.

which is likely, then the totals shown in the table for 1975 would increase, the totals proposed for 1976 would decrease by a similar amount, and the total increase being proposed for the agency would shrink considerably.

In any event, Congress is likely to reorder the priorities in ERDA's budget a little, certainly by increasing non-nuclear appropriations and probably by reducing the amounts proposed for fission technology as well.

Even according to the OMB's analysis, however, the total amount of money that President Ford has proposed to spend on energy research and development in 1976 is more than double the total spent in 1974—\$1,663 million compared with \$833 million. A question that has not been raised too openly is whether that huge surge in funds can be usefully absorbed in such a short space of time. □

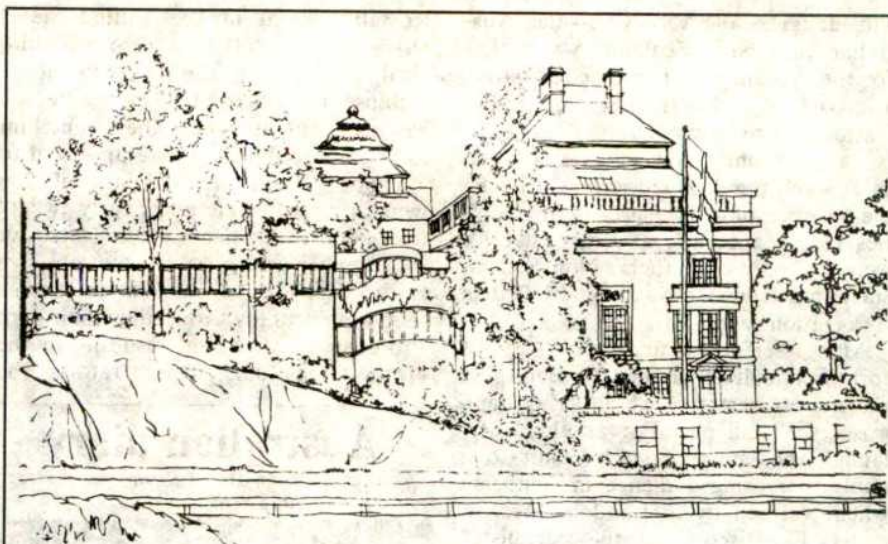
Mining company claims sea bed

from Peter J. Smith

THOSE who see exploitation of the ocean floor as a potential source of world conflict will take little comfort from a paid advertisement which appeared last week in *The Times*. For in the small-print Public Notices column, Deepsea Ventures Inc., of Delaware and Virginia, announced that "it has discovered and taken possession of . . . a deposit of North-east Pacific Ocean seabed manganese nodules."

The section of the sea bed over which the company claims "exclusive mining rights" is the area bounded by the latitudes 14° 16'N and 15° 44'N and longitudes 124° 20'W and 127° 46'W—a region lying in international waters between the Hawaiian Islands and mainland North America. Within this zone Deepsea Ventures intends to "develop, evaluate and mine the deposit and to take, use, and sell all of the manganese nodules . . . and the minerals and metals derived therefrom". As a form of "diplomatic protection and protection of investment" the company also "requests and requires [sic] states, persons, and all other commercial or political entities to respect the exclusive rights asserted".

Deepsea Ventures says that the validity of its claim is established "under existing international law as evidenced by the practice of States, the 1958 Convention on the High Seas, and the general rules of law recognised by civilised nations". In fact, the legal position is far less certain than that statement would suggest; the 1958 convention, for example, made no specific mention of any freedom to exploit the deep sea bed because at that time



NOT all of Pugwash's cries fall on deaf ears. A little known Swedish listener has offered to give substance to one of Pugwash's recent pleas: for an international institute to carry out advanced theoretical scientific studies into energy and ecology problems.

The pricked ears belong to Kjell Beijer, a Swedish businessman who last year set up the Kjell and Märta Beijer Foundation for the advancement of scientific research in Sweden. The foundation has decided that its first project will be the financing of the new institute, and is to donate Sk12 million (about \$3 million) over an initial period of 10 years for running costs and SK3 million for the construction of a building—to be attached to the Royal Swedish Academy of Sciences—in which the institute will be housed. The building should be ready by the end of 1976.

The institute will have close links with the academy in more ways than one. The academy is to be responsible for its establishment, and is at present working out the details of its organisation with the Royal Swedish Academy of Engineering Sciences. The main idea is clear: the institute should be shaped along the same lines as the Stockholm International Peace Research Institute (SIPRI). An international board of scientists will be responsible for the overall planning of the work, and a director (not yet appointed) will run day-to-day activities, make suggestions to the board about new projects and carry on his own research. The actual projects will

Architect's sketch of the Royal Swedish Academy of Sciences building showing how it will look when the new institute is attached (at left).

be carried out by 10–15 scientists, including guest researchers who will be invited to work on problems in specific areas. Four areas have been suggested so far:

- Surveys of energy resources, including new primary sources, such as solar, geothermal and fusion energy.
- Energy and economic development, taking into account the energy problems of developing countries.
- Energy and environmental effects, including the protection of the atmosphere against thermal, chemical and nuclear damage.
- Energy in relation to other resources, for example, minerals, water and food.

The focus will be purely theoretical. No experimental work is planned. This follows from the conception of the institute as a collector, analyser and disseminator of information to governments and the growing number of groups around the world who have started to take an interest in energy questions. The most visible of the institute's activities will be conferences, workshops, symposia and publications.

And when will work begin? With luck before the building itself is ready. But even if it takes until 1977 to work up full steam, the scientists can be sure that there will still be lots of energy problems left for them to solve.

Wendy Barnaby

exploitation was considered impractical.

In effect there is no international law on this matter—an omission which gives equally eminent authorities the opportunity to disagree about whether or not any form of occupation of the ocean floor is allowed. Last year's Law

of the Sea Conference in Caracas failed to resolve the question. It rather looks as though Deepsea Ventures has timed its announcement to concentrate the minds of the delegates when the conference reconvenes in Geneva next month. □

THE congress of ANZAAS—the Australian and New Zealand Association for the Advancement of Science—drew a crowd of 2,500 to the Australian National University campus in Canberra last month. Not as good a crowd as at some previous gatherings said the old-timers, and the spread-out facilities led to a feeling at times that we were the only ones there. Nonetheless, such an attendance was huge by British Association standards.

Many scientists find this sort of forum difficult to handle. For one thing there is uncertainty about who comprises the audience. For another, why launch the latest research result to a dimly discerning audience in Canberra when you can fly to London, Moscow or San Francisco to tell the specialists? This undoubtedly largely accounts for the relative poverty of the physical science section, but other sections gain by their very breadth of appeal. History, geography, education, industrial relations and economics all find a home in ANZAAS and, it is said, grow. But food science and nutrition attracted most attention this year. This could be seen partly as a reaction to the world food situation and partly as a move towards whole-animal (and especially whole-man) science, but maybe also as a reflection of an uneasiness amongst Australians that their diet encourages obesity. And all the signs are that obesity is on the increase.

The theme for the conference, "Science, Government and the People" was largely pursued through nine symposia inconveniently scheduled so that nobody could attend more than three. The quality was very uneven, but probably those who attended diligently emerged with a broader understanding of the scientific community and its external interactions. A national project for the public understanding of science 'warts and all' was one practical proposal that emerged.

It was encouraging that Mr Bill Morrison, the Minister for Science, and Mr Tony Street, Opposition spokesman, were so much in evidence and participated actively in the proceedings. Would that a few politicians in other countries made themselves visible and accessible in such gatherings, if only because most scientists view science policy as something remote and irrelevant and could profitably learn what the politicians think about it.

Police vans flanked the entrance to the Academy of Science for the symposium on scientific and technological research in universities and colleges of advanced education. Hardly an explosive subject, but the presence of Professor Roger Russell (Vice-Chancellor of Flinders University) guarantees a demonstration these days. Professor

Russell worked in the United States on various projects related to psychological warfare for the Department of Defense and according to some he still has involvements which they want him to disclaim publicly. There appeared to be about 20 in the audience of 200 who came to heckle and nearly twenty television technicians whose intrusive role in triggering confrontation was fairly obvious; but after some tough-mindedness from the chairman, Professor J. M. Swan, resulting in the ejection of four demonstrators, the

Australian diary

from David Davies
and Peter Pockley



Fly me

meeting was uneventful apart from some fairly conventional interruptions. Maybe three and a half hours on the binary system in advanced education was more than any demonstrator could bear. Maybe they feared for the safety of their tape recorders if ejected. Or maybe it was the fear of being dumped in the moat surrounding the academy.

With the exception of the Pharmaceutical Sciences Section which regularly brings a team of overseas experts to Australia for these congresses through direct funding by drug companies, ANZAAS meetings seldom attract contributors from overseas to present papers of international significance. It was therefore all the more notable that Dr R. J. Murgatroyd, Chairman of the British Meteorological Office's Committee on the Meteorological Effects of Stratospheric Aircraft (COMESA), chose ANZAAS as the venue for a major announcement.

Australia has been the scene of extensive public debate on the alleged deleterious effects of the SST aircraft, particularly Concorde. Various airlines want to fly it to Australia from Europe and America (the London-Sydney trip could be cut by half to 12 hours

or so). An environmental bandwagon against Concorde has had considerable political effect over the past three years. Despite an Academy of Science report which cautiously cast doubt on the validity of theoretical projections by American and Swedish chemists, the Australian government has so delayed confirming orders for Concorde that most people think the deal is off.

Dr Murgatroyd's conclusions were clear—several hundred Concorde would not reduce the stratospheric ozone concentration by more than 1%, 1,000 Concorde would cause a reduction of about 3%, and 2,000 Concorde about 6%. Because of their higher operating altitude (20 km, as against 17 km for Concorde) and their higher engine emissions by virtue of a greater size, American-designed SSTs could produce roughly twice the effect. Climatic changes brought about by Concorde would similarly be minute, several hundred Concorde resulting in a variation of less than 0.1 °C. Formation of condensation trails would be unlikely to lead to anything more than minor increases in stratospheric clouds, and these only in equatorial regions and polar areas in winter. All effects are well within natural variations (for example stratospheric ozone has been found to vary by $\pm 6\%$), and the effects of subsonic aircraft may be greater in some cases.

The conclusions of COMESA according to Dr Murgatroyd, are consistent with the results of parallel American and French government research programmes, the American research having now been published as well. The greatly increased confidence of their predictions is accounted for by several factors, all linked with the availability of massive and reliable data on natural atmospheric processes, engine emissions, numbers of aircraft, flight routes, photochemical reactions and mathematical modelling.

Dr Murgatroyd considers the ozone in the stratosphere to be under greater threat from the possible deleterious effects of freon gases from pressure pack sprays and refrigeration equipment. First rough calculations suggest a reduction of 1% in O_3 at present levels, but in 15 years the reduction could go up to 5%.

The campaign against Concorde and the fears about damage to the Great Barrier Reef by *Acanthaster planci*, the so-called Crown-of Thorns starfish, rank equally as the biggest single environmental issues raised in Australia. Over the past five years or so, the apparent southward march of the starfish along the 2,000 kilometres or so of the largest string of coral reefs in the world has retained the interest of public and politician alike. The campaign for direct action to "save the

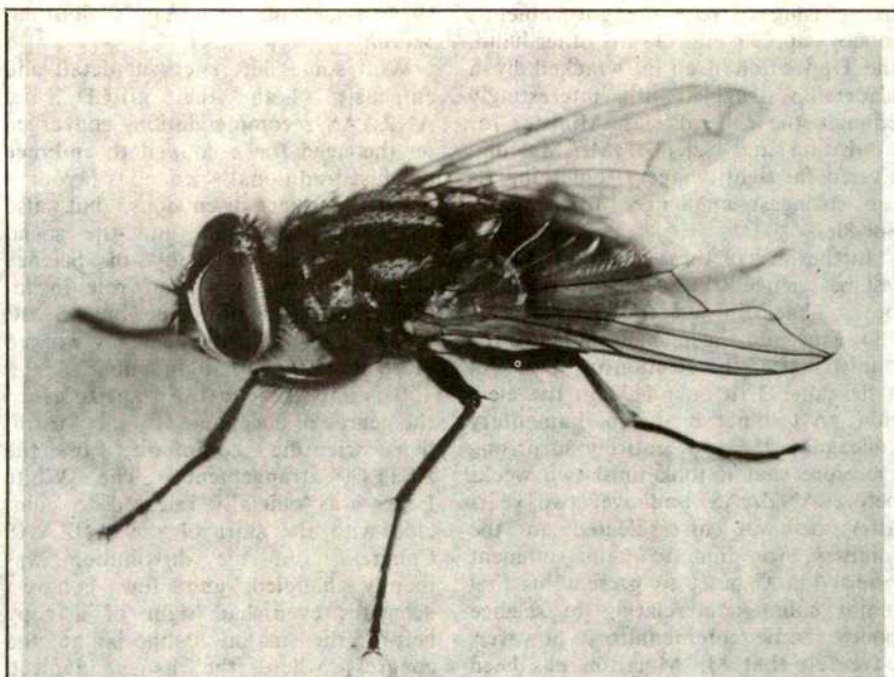
reef" has been led by some zoologists in Queensland, and although nobody has doubted the existence of a real problem, there has been considerable disquiet among less vocal scientists regarding some proposed solutions (for example, direct killing of starfish *in situ*, using teams of divers) and the emotionalism which crept into the publicity.

Nonetheless, the Australian government established a special Advisory Committee for Research on the Crown-of-Thorns Starfish to administer a research fund. \$A330,000 (Aust) was allocated in the first three years of the committee's existence (1972-4), and the original limited programme has been extended for a further year with \$150,000. More government support went into an ambitious survey of 25 reefs in the Great Barrier Reef geological province last year, using ships and divers from the Royal Australian Navy. Mr Richard Kenchington, research fellow of James Cook University, Townsville, who co-ordinated the survey, reported at the ANZAAS Congress that the numbers of starfish on 14 of these reefs were negligible. This, however, has not led to complacency, for the threat to research areas covering the whole range of marine science, such as that at Heron Island, and the economically important tourist reefs is still keenly felt.

Mr R. G. Pearson, of the Department of Primary Industry, Queensland, reported some encouraging results on his surveys of the rate of regeneration of coral reefs after the 'wave' of starfish has passed. Soft corals appear to re-establish themselves in a few years. This kind of research and that concentrating on the largely unknown biology and behaviour of the species were strongly commended for future starfish research by a panel of influential scientists at ANZAAS.

Among other hard-hitting speakers, Dr Frank Talbot, Director of the Australian Museum, Sydney, said the starfish "plague" is not a real problem, is not man-induced and should not be supported by a special research fund. Others welcomed the strategic significance of the public support for the starfish research programme in that it focussed attention in Australia on the long neglected marine sciences and on the great ignorance of the Great Barrier Reef. Now that the ambitious Australian Institute of Marine Science is under way, the general consensus of speakers seemed to be that the days of a research programme relating to a single species and absorbing a substantial proportion of marine research funds should be numbered.

● The announcement at the congress of



THE most recent British Airways advertisements for Australia show a gnarled man (opposite) wearing a hat around the brim of which dangle a dozen corks on the end of pieces of string. The purpose of this hat has been widely debated in Britain, and the British visitor will find even more mystification when he lands at Canberra to be greeted by what appear to be royal waves from the locals. The explanation of both these phenomena is the ubiquitous bush fly, *Musca vetustissima*, and no tour of the capital is complete without a visit to the entomology division of the Commonwealth Scientific and Industrial Research Organisation (CSIRO) to find the latest on biological control of this pest.

Dr D. F. Waterhouse, director of the division, wrote in *Nature* in 1973 (246, 269) about the dung problem in Australia. Thirty million cattle each produce twelve dung pads a day, and the means of their disposal are on the whole lacking in Australia. Whereas in other countries various dung beetles get to work in their tunnelling and ball-rolling as soon as the dung hits the ground, in Australia it can sit there for years, eclipsing signi-

ficant areas of land, leading to grass that cattle won't eat and providing an ideal breeding ground for flies.

Gradually the problem is yielding. Sterilised imported dung beetle eggs from Africa have been used for several years to generate huge populations of coprids which have been spread around northern Australia with some success. It has become clear, however, that it is necessary to supply a variety of species (in Africa there are at least 2,000) if the attack is to be entirely successful.

And will the farmer accept an intrusion of scientists on his cow patch? Yes, says Dr Waterhouse, if things are done through the proper state channels.

Not all the division's work is unmitigated dung dispersal. There are new and highly successful techniques for eliminating insects from grain storage without resort to malathion. There are attacks on the skeleton weed by the use of fungi. There are genetic assaults on the sheep blowfly. There are even new ideas for fruit tree pruning. The returns on investment are potentially high as some pests cause tens of millions of dollars worth of damage every year.

the terms of reference for the Australian Science and Technology Council (ASTEC) does not guarantee the rapid formulation of Australia's first clearly enunciated science policy. The main danger to ASTEC is precisely that which afflicted the defunct Advisory Committee on Science and Technology, appointed by the last Liberal Government in 1972: the threat of imminent political change.

Since Mr Whitlam's Labour Party

was re-elected in May 1974, the government has lost its public support to a quite remarkable degree. Inflation hit Australia hard only in the past eight months and is now running about 20% a year. Unemployment has risen to a near-Depression level of almost 4%, an intolerable situation for a socially oriented Party. There is daily talk about whether the Opposition-dominated Senate will force another election this year, as they did last year

by refusing to pass the government's money supply plans. On the other hand, the Opposition itself is wracked by a leadership struggle, with, interestingly enough, the Liberals' last Minister for Education and Science, Mr Malcolm Fraser (a right winger), emerging as the strongest challenger to Mr Bill Snedden.

In the face of these running political issues, science still seems pretty small beer. The Minister for Science, Mr Bill Morrison, just made it into the ministry after the election in May 1974—he came 27th out of 27 in the elections to Cabinet by his parliamentary colleagues. It is not entirely surprising therefore that it took until two weeks before ANZAAS, and over two years after he was first elected to the ministry, for him to gain sufficient priority in Cabinet to present his first major submission relating to science policy. Some commentators, however, have felt that Mr Morrison has been content to let things drift for, apart from the consumer cause which he has espoused with some energy, he has seen little political advantage to be gained in science.

The two-year wait has been a frustrating one for those Australian scientists who had been looking to Labour for action. Labour had a science policy in its Party Platform for some time in contrast to the Liberals' steadfast refusal to acknowledge the very possibility of, or need for, a coordinated approach to science. The year 1974, however, brought forth a rash of documents and discussions which have led to the final formulation of ASTEC, and no interested party can now complain that their voice has not been heard. There are some, though, whose voice may have been heard but not heeded. Notable in this category must be the Department of Science whose heads can hardly be happy with the statements in the White Paper launching ASTEC which show that the department's role in recommending policy to the government will be subordinate to that of ASTEC.

Mr Morrison's major move towards gaining a consensus about a science council was the invitation to the OECD to carry out one of its independent surveys of science policy. This study, under the leadership of the redoubtable Dr Alexander King, was carried out early in 1974 and led to a preliminary report by the OECD and one of their "confrontation" meetings in Paris last autumn. Meanwhile, ANZAAS had established a Science Policy Commission under the chairmanship of Professor Sol Encel, a sociologist at the University of New South Wales, who is one of Australia's few professional students of science policy. Its report was published in the November

1974 issue of ANZAAS's journal, *Search*.

With some differences in detail and emphasis, both the OECD and ANZAAS recommendations converged on the need for a council to embrace not just traditional science, as favoured in the earlier discussions, but also technology, medicine and the social sciences. The Academy of Science also played an important role in influencing the final shape of ASTEC but, as is its wont, the academy worked more in private than in public.

It is impossible at this stage to assess the degree of acceptance by the Australian scientific community of the ASTEC arrangements. The White Paper was officially released to coincide with the start of the ANZAAS Congress, but the distribution was ineptly handled and few congress delegates even had sight of a copy before the major symposia at the congress where the issues involved were discussed. A meeting to discuss the ANZAAS report mustered a mere 70 in the audience.

But it is already clear that Mr Morrison's proposals (he claims to have largely written the White Paper himself with help from his staff—there is no evident love affair between the minister and his public servants) comprise a minor political victory in three areas, all of which will be welcomed by Australian scientists. These involve the terms of reference and powers of ASTEC to encompass not only science, but also technology, the medical sciences and defence science (within the limits of security). To the disappointment of some, ASTEC's role in relation to the social sciences is not at present intended to extend beyond the interaction of the social and natural sciences, as in multidisciplinary studies of complex problems.

The political victory comes in that funding of research and development technology, medicine and defence have come exclusively under ministries other than Science, and this has contributed substantially to the fragmentation and lack of direction in Australia's overall science effort. Proposals for the integration of these disparate elements will have to be formulated with great

bureaucratic and political skill by the members of ASTEC, and therein will lie a major test of its potential influence in the science scene in Australia.

The Labour Party's Platform had advocated that the members of a science council be elected by the scientific community; this has been abandoned in favour of the traditional method—appointment by the Minister, who significantly for the status of ASTEC will formally be the Prime Minister. ASTEC will be unusually sensitive to the quality of its first appointees, and further significant commentary on it may have to await that announcement.

● Gough Whitlam himself gave a paper at the 'Grand Symposium' on the final night. Recently returned from extensive foreign travels, he shared the platform with the Presidents of the Academies of Science, Social Sciences and the Humanities in an evening of remarkably good public speaking. His speech was mainly directed at the rationale behind the science policy White Paper, but he surprised the audience by taking the floor again after the main speeches to deal firmly with issues raised—particularly some questioning of his uranium policy and some remarks about trying to keep academics, particularly in the humanities, from emigrating. Professor J. Passmore (Academy of the Humanities) had been bemoaning the poor quality of Australian libraries and the great delays in the arrival of journals.

Nowhere on his travels, Whitlam said, had anyone suggested that the uranium should be kept in the ground, although he was fully aware of proliferation problems and hoped to "do something" about them.

On emigration, he professed no concern. Maybe the scholars move away but the practitioners come back to play, sing, paint and so on. Not quite the same thing, muttered the audience as they left, dazed that even a mild bit of politicking had entered their sedate arena. And there wasn't a glimmer of a response to Passmore's gentle suggestion that the government might air-freight journals from Europe and North America. □

Must new universities be poor relatives?

● In 1946 there were 25,600 students in six Australian universities. In 1972 there were 128,000 in eighteen. But there is a failure rate of 30% and many academics speak with concern of the quality of staff in some of the newer universities. If these universities are not to be seen as poor relatives, they face a long haul of consolidation. There could be a major role for CSIRO in this. The OECD report speaks of a need for greater mobility amongst Australian scientists and it may be that the new universities, armed with this report, should seek strengthening of their staff through more direct collaboration and exchange with CSIRO.

A searching look at Israeli science

from Nechemia Meyers

AN extraordinarily incisive and explosive 71-page report on the interaction between Israeli industry, science, universities and government was released this week by the Jerusalem-based National Council for Research and Development after being kept under wraps for two years. The report, submitted to the National Council for Research and Development (NCRD) in December 1972 by Dr S. Wald, a well known OECD economist and expert on science policy, notes that the country invests very substantial amounts in research and development (2.5% of its GNP) and that its research is of very high quality. Nevertheless, Wald charges, Israel's policy for research and development lags behind that of western Europe and North America "perhaps more than it did 20 years ago".

The factor primarily responsible for this situation, in Dr Wald's opinion, is an insufficient emphasis on applied research. "Israel," he says, "appears to be the last bulwark of the old faith which puts theory and pure science above practice and applications".

Wald caustically suggests that Israeli professors, to whom he attributes great influence, "may have greater respect for discovery than for development because few have had experience in development work and thus can hardly be expected to realise that the discovery of new material is sometimes intellectually less demanding than the discovery of methods to produce that material in tons and for an acceptable price".

Government ministries, Wald argues, have little more understanding of the situation. In the words of the report: "they act as if once a great 'discovery' is made its 'development' will be routine work and can more or less take care of itself".

Even industrial companies "have interiorised the prejudices of which they are the main victims", Dr Wald believes, adding that men who in Europe would be called "research directors" and in the USA "vice-presidents for research" are in Israel termed "development directors". This suggests that "nothing or almost nothing of their work would qualify as 'real' research".

Dr Wald reports on conversations with Israelis who link the country's single-minded dedication to pure research with Jewry's traditional emphasis on learning for its own sake. The image of Einstein, who changed the world and gained immortality with the stroke of his pen, was also invoked. This image,

Wald says, "has perhaps left a more disastrous mark on the minds of young people in Israel than in any other country".

Credit must certainly be given to the NCRD, which is part of the Prime Minister's Office, for publishing a document which declares: "No government in the Western World has so much concentrated economic and industrial power, yet none uses it so little to define and to achieve precise technological goals involving industry and universities".

It is perhaps not without significance that the document, which takes the government to task for failing to deal with questions of science and technology on the Cabinet level, was released only after the belated appointment (last month) of a Ministerial Committee on Science and Technology.

One goal of this new committee will certainly be to coordinate existing research programmes of government ministries which, Wald says, "to some extent still consider each other as competitors and not as complementary instruments of one and the same nation". His report cites the example of a project which the Ministry of Commerce and Industry approved on scientific grounds, but would not finance because telecommunications "belonged" to the Ministry of Posts and Communications (which at that moment had no applied research fund).

Marketing is much underrated in Israel according to Wald, who found it regarded "as a second-rate propaganda job", rather than as a suitable occupation for skilled scientists and engineers. The OECD economist also questions the Israeli tendency to look for large American corporations to serve as partners and marketing agencies for local industry. These big companies, he warns, are likely to view Israelis as very minor partners who can be dropped quickly if other considerations arise (a need to cultivate the Arabs, for example).

Dr Wald suggests that Israel should model her technological development programmes on those of small Western European countries like Holland, Switzerland and Denmark rather than on the USA. He points out that, like Israel, they have meagre natural resources and that their relative prosperity has been achieved by concentrating on the development of a few quality products, with which they are capable of competing on the world market. Israel's key products, he says, might come from the electronics and aeronautics industries, and more particularly from a few companies, like Israel Aircraft Industries, with sufficient turnover to finance a serious programme of research and development.

Western European countries accord

applied research and engineering higher prestige than they enjoy in Israel, Wald notes, mentioning in this context the fact that these nations sometimes create highly prestigious national academies of engineering science, a parallel to their science academies. Moreover, their universities are quick to meet local industrial requirements. When the Swiss watch industry was in trouble, Wald recalls, the university in Neuchâtel set up a faculty to train watch-making engineers "without worrying whether watch engineering was a recognised engineering profession, was a 'science' that could lead to publications or was represented at MIT".

Wald notes that Israeli professors have in recent years stressed their commitment to industrial development, but wonders if they are indeed prepared for a drastic change in science policy. The fact that Israeli industrialists still receive letters of recommendation from professors stating that a particular student is not quite good enough for PhD work but would make an excellent researcher in industry "speaks louder than public declarations", Dr Wald says.

With all his criticism, the OECD economist remains optimistic about Israeli's industrial future. "A visitor", Wald observes, "has the almost daily impression of an exceptional reservoir of will and talent which could be organised with more efficiency and profit. Many of the ingredients which make for a powerful industry-technology-science interface can be found in the country. Like pieces of a puzzle, they lay there—some fit together already, others are still in disparate order awaiting the hand that will push them together". □

First catch your bear

THE Soviet Ministry of Agriculture has drafted a special programme, within the framework of the existing international agreement, for the protection of Polar Bears. Accordingly, an expedition is shortly to leave for Wrangel Island, which has the largest bear colony in the Soviet Arctic.

The tagging of adult bears and their cubs is to take place in March and April, when the bears bring their cubs out to bask in the sun. The procedure, it is announced, will take the form of painting a number in red on the backs of the bears and the insertion of metal rings in their ears.

The *Novosti* press release announcing this expedition is headed optimistically "Polar Bears no longer threatened with extinction". A happy prospect for the bears—but perhaps less so for the painters and ear-ring fitters. One hopes that the expedition will be equipped with a supply of stun-guns. □

correspondence

Czech mathematician

SIR,—On January 10, 1975, a petition was submitted to the Czech embassy in the Netherlands, concerning the position of the Czech mathematician and computer scientist Professor Karel Culik. The petition was worded as follows:

"The undersigned mathematicians and computer scientists are deeply concerned with the present position of Professor Karel Culik. Although his work has done much for the world-wide renown of Czech mathematics he is at the present time prevented from taking part in normal scientific activity; he has no position in his own country and at the same time cannot obtain permission to accept the offers he has from universities in foreign countries. The undersigned strongly urge the Czech authorities that Karel Culik be allowed shortly to pursue his career as a researcher and a scientist."

The petition was initiated by the European Association for Theoretical Computer Science, and signed by 238 scientists from 12 countries.

Yours faithfully,

J. W. DE BAKKER

(Vice-President, European Association for Theoretical Computer Science),
Amsterdam, Netherlands

Consultancy

SIR,—Two correspondents (December 13 and January 17) have recently criticised the way in which university staff misuse the facilities at their disposal by carrying out consultancy work.

There are other ways in which university staff take advantage of their position, particularly their flexible hours of work. Many for example mark GCE examination papers, or act as GCE examination supervisors, or as tutors at Open University Summer Schools. For these duties they are either paid or receive generous expenses.

Yours faithfully,

DERRICK BAXBY

University of Liverpool, UK

Golden handshake

SIR,—You make the proposal (January 10) that the golden handshake is the solution to the job stagnation problem in British universities. As a younger lecturer (holding an appointment in dental biochemistry) I feel such a measure would only make the situation worse. Senior experienced staff

would be removed and this would only add to the administrative duties and frustration of younger colleagues at the most intellectually fertile period of their careers.

An alternative solution to the problem would be the introduction of opportunities for lecturers who wish to retrain for other professions in the middle of their careers, with financial assistance during this period of training comparable to their existing salaries. Those in their late thirties still have 60 to 70% of their active careers before them.

The people concerned are first-rate men and women, who have reached their present positions in a highly competitive and selective field. Prior experience in academic life could make an important contribution to a subsequent career in a different profession. For example, the experience and knowledge gained by a biochemistry lecturer would be an invaluable asset to someone subsequently studying medicine.

Mid-course retraining is already practised in industry; it could well offer the solution to the academics' problems too.

JOSIE A. BEELEY

University of Glasgow, UK

For those in peril

SIR,—Your correspondents in this series (October 18, November 22, November 29) do not distinguish clearly between the scientific and political aspects of pollution. Peter J. Smith's statement that "asbestos products (and thousands of other equally or more dangerous substances) are here to stay" is not a law of nature, it is a political statement. It indicates that we are prepared to pay, wittingly or unwittingly, with our own, or other people's tissues for the goods and services that these substances offer.

To be able to say under what conditions they are to stay, the scientists will have to tell us about the medical effects of existing pollutants in industrial environments and in the general environment, whether the effects are dose-related, how concentrations can be monitored, and how potential pollutants can be recognised—all difficult, expensive and time consuming problems. But the medical scientist cannot judge this—to him all avoidable tissue damage is totally abhorrent. 'Public opinion' has to decide how much discomfort or loss of years an individual

must pay.

How can the cost or risk for each pollutant be presented intelligibly for the public to judge its acceptability? Setting out the expected health effects, as for SO₂ and smoke, is one way. But concentration-time scales would incorporate commercial and political realities. LD₁₀₀ (concentration X for N years) is bad for business (and votes). A lower product for ill-health may be commercially acceptable in terms of lost man hours or compensation, but becomes less acceptable in the context of individual workers.

But the data must be sound. Unsound scientific or political activism could so limit industrial or daily activity that ill-health from pollutants could become replaced by poverty.

W. F. WHIMSTER

London SE3, UK

The economics of recycle

SIR,—In order to put recycle in a proper perspective (January 17), it is necessary to remember the following factors:

The future availability and price of key raw materials, as well as of oil, have been placed in doubt by the events of the past year. These events have emphasised the need to conserve our national resources. Recycle, or reuse of materials, is an important aspect of the proper management of these resources. It is, however, but one aspect and should be operating along with other measures such as improved design and reduction in waste both in manufacture and use.

Recycle is only viable for those materials which can be collected, reprocessed and made into products which can be sold at competitive prices. The problems are therefore basically technical and economic.

T. S. McROBERTS

Wolfson Recycle Unit,
Queen Mary College, London, UK

Nutrition

SIR,—I would be grateful if you would make it clear that the views expressed by Mr John Rivers in your article entitled "Between combine harvester and ribosome" (January 10) is an expression of his own views. The article should not be regarded as an official pronouncement of this institute.

L. G. GOODWIN

Nuffield Institute
of Comparative Medicine,
Zoological Society of London, UK

news and views

Molecular evolution and the age of man

from John Maynard Smith

It is always intriguing when scientists with different backgrounds and training apply their different techniques to the same problem, and come up with totally different answers. This situation has now arisen in the study of human evolution. The traditional palaeontological approach suggests that the lines leading to man and to the great apes diverged at least 15 million years ago, and perhaps longer; biochemical evidence has recently been interpreted as showing that the time could not have been more than 5 million years.

At first sight, a comparison of proteins of different species seems just one more phenotypic comparison providing information similar in kind to that provided by a comparison of bones or teeth. This is not so. Knowing that the primary sequence of, for example, the insulin A chain in guinea pig and coypu differ by six amino acids, we can assert that at least six gene substitutions must have occurred in the relevant gene along the lineages leading from the common ancestor to the two species. A knowledge of the genetic code tells us that two of these substitutions could not have taken place in a single step, and hence that at least eight substitutions have taken place. Of course, these are minimum estimates. If the same changes have taken place in both lineages, or if some sites have changed several times, we shall underestimate the actual number of changes. This difficulty is a real one, but is probably less serious than might have been expected. A comparison of two proteins does enable us to estimate the number of gene changes that have occurred; we have no idea, even to an order of magnitude, of the number of gene changes responsible for the difference between the canine teeth of ape and man.

This method, combined with absolute dating from the fossil record, has led to some generalisations. Different types of protein evolve at very different rates. For example, cytochrome C evolves rather slowly, and fibrinopeptides (small peptides cleaved off fibrinogen when clotting occurs) very fast. A given class of proteins, however, evolves at a rather constant rate in different lineages. The exact degree of uniformity has been hotly debated, partly because of its relevance to the

'neutral' versus the 'selective' interpretation of protein evolution. The neutral theory asserts that most (though of course not all) amino acid substitutions which occur in evolution are selectively neutral. It is a prediction of this theory that the rate of evolution of a given class of protein should be uniform; hence the heat. Some striking exceptions are known; for example, insulin is remarkably uniform among mammals, except among hystricomorphs (a group of rodents which includes the guinea pig and coypu), which differ from each other and from other mammals by ten or more substitutions. A number of less striking discrepancies are known, but one is left with the impression of a surprising degree of uniformity of rate, at least when compared to morphological characteristics.

Unfortunately, it takes a lot longer to sequence a protein than to measure a bone (and longer still to discover the tertiary structure of a protein, which is a necessary first step if one is to make functional sense of the amino acid substitutions). This difficulty can be partly overcome by use of immunological techniques. A protein from species A is purified, and antiserum to it prepared. The 'microcomplement fixation' technique is then used to compare the extent of the reaction between the antiserum and the original protein from species A with the reaction with proteins from other species B, C, D and so on; it is a major convenience that the latter do not need to be purified. In this way, the 'immunological distance' between proteins can be measured. The technique has been extensively used in evolutionary studies by Sarich, Wilson and their colleagues. The protein used has been albumin; this is a large protein which evolves rather rapidly and so provides a lot of information. There is evidence, for albumin itself and for other proteins, that immunological distance measured in this way is linearly related to the number of amino acid substitutions. The relation is not exact, but it is close enough to make immunological distance a reliable guide to number of genetic changes.

The rate of evolution of albumin in different groups of vertebrates is apparently rather uniform (very approxi-

mately, two amino acid substitutions per million years, in a peptide 380 residues in length). As mentioned at the beginning of this article, the most controversial finding (Sarich and Wilson, *Science*, **179**, 1146; 1973) concerns the evolution of man. Three lines of biochemical evidence (immunological distance; electrophoretic comparisons of a number of proteins; direct sequence data on a few proteins) show that man and the great apes, and in particular, man and chimpanzee, are remarkably similar. These data suggest a time of divergence of perhaps three million years ago, and certainly not the 15–20 million years conventionally accepted. The obvious response has been that protein evolution has been particularly slow since the divergence, perhaps because of the long generation time. Wilson and Sarich retort that there is no evidence in favour of such a view. They argue that absolute dating of common ancestors from the fossil record is unreliable, but that the relative times of branching points are reasonably certain. For example, the common ancestor of man and tree shrew (*Tupaia*) is later in time than the common ancestor of these two and the bear or hyaena. Therefore, if protein evolution in the human lineage has been slow (because of long generation time or for other reasons), then the difference between man and bear should be less than that between tree shrew and bear. In fact, judging by immunological distance, the opposite is the case (although the effect is small). From this and other arguments, they conclude that protein evolution has not slowed down in the human lineage, and that the evidence cannot easily be reconciled with a divergence time greater than five million years.

This conclusion has not recommended itself to palaeontologists. Australopithecines, regarded as well on the way to being men, date back at least three million years. *Ramapithecus*, believed also to be on the human lineage, lived 12 million years ago. Dryopithecines, very similar to modern great apes, date back 20 million years. In the face of this direct evidence, it is hard to accept Wilson and Sarich's conclusion. We ought, however, to remember that *Ramapithecus* is, after all, only a few jaws and teeth, and that one school

of anatomists (noted, it is true, more for panache than perspicacity) still argue that *Australopithecus* is as close to the great apes as it is to man.

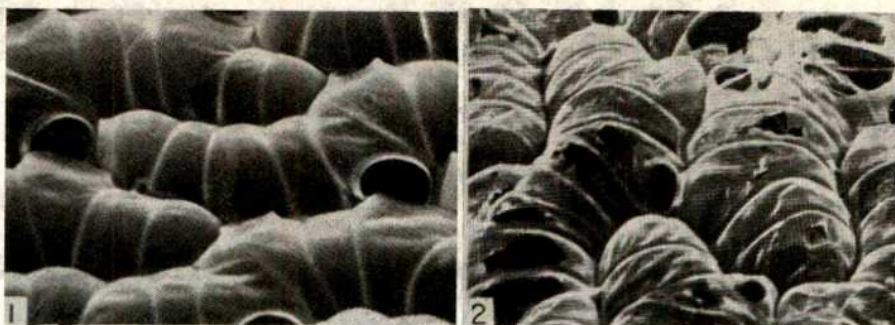
Equally surprising, but less controversial, conclusions have emerged from a comparison of albumin evolution in frogs and mammals. The rate of evolution of albumin has been very similar in the two groups, despite the much greater rate of morphological evolution in mammals (the first fossil frogs are 150 million years old). Wilson, Maxson and Sarich (*Proc. natn. Acad. Sci. U.S.A.*, **71**, 2843; 1974) have compared the albumins of 31 pairs of mammal species and 50 pairs of frog species which can produce viable hybrids. The average immunological distances are three and 36 respectively, implying that a tenfold greater degree of differentiation in protein structure is compatible with hybridisation in frogs than in mammals. The authors discuss the possibility that this is because of immunological reactions between mother and foetus in mammals, but give reasons for preferring an alternative explanation. They suggest that hybrid embryos die because of differences in gene regulation between the parental species, and that, relative to evolution of structural genes, gene regulation evolves more rapidly in mammals, associated with their more rapid morphological change. In a later paper (*Proc. natn. Acad. Sci. U.S.A.*, **71**, 3028; 1974) the same authors point out that chromosome evolution (as judged by changes in numbers of chromosomes and of chromosome arms) has proceeded more rapidly in mammals than in frogs and suggest that structural changes in chromosomes are associated with changes in gene regulation.

Decomposition in peatlands

from Peter D. Moore

MOST ecosystems in which production is in excess of respiration and decomposition accumulate the surplus energy-rich material as living biomass; peatland ecosystems are peculiar in that their excess is deposited as litter and accumulates as peat. In some peat-forming ecosystems, such as reedswamps, net primary production is high, but in those exhibiting the highest rates of peat accumulation, primary productivity is quite low. In such instances it is reasonable to assume that peat accretion results from a depressed decomposition rate rather than an inflated productivity.

Because mires hold many attractions for biologists concerned with the evaluation of energy and dry matter budgets for ecosystems, and also because peat is



Sphagnum magellanicum branch leaves, convex surfaces showing hyaline cells with supporting bars of secondary wall material and bordered pores. 1, green, living leaf taken from top 0.5 cm of plant; $\times 560$; 2, leaf taken from peat at 10 cm depth, showing partial decomposition of hyaline cells; $\times 460$.

an important natural resource in several countries, it is not surprising that a number of attempts have been made to develop mathematical models of peat-accumulating systems. The efficiency of any ecosystem model is limited by the reliability of the raw data used in its construction and, in the case of peat-forming systems, the most elusive information is that related to decomposition.

It is usually assumed that the loss of organic matter decreases exponentially with time (for example, Gore and Olson, *Aquilo Ser. Botanica*, **6**, 297; 1967). Although such an assumption is reasonable, its confirmation, and a precise knowledge of long-term decay rates for different materials, are extremely difficult to obtain. Clymo (*J. Ecol.*, **53**, 747; 1965) approached the problem by burying nylon mesh bags containing known quantities of *Sphagnum* moss at various depths in the peat. He demonstrated that losses were far higher in the superficial layers of peat than from deeper down, where conditions were permanently anaerobic. The disadvantage of such a method is that in natural circumstances the material entering the anaerobic zone would have already undergone some degradation during its period in the upper peat layers; on this basis one might expect even lower levels of anaerobic decomposition than Clymo's data suggest.

Direct observations on bracken decomposition over a period of years led Frankland (*J. Ecol.*, **54**, 41; 1966) to believe that the course of decomposition was essentially linear, and Reader and Stewart (*Ecology*, **53**, 1024; 1972) have therefore used this assumption in their model of peat accumulation in Canadian muskeg. They use decay rate figures from various plant materials which were exposed in bags for a period of a year. But the apparently linear decay observed by Frankland may have proved to be exponential if observation could have been continued over very much longer periods of time, and the assumption of linear decay is not generally favoured.

Two recent studies of peatland decomposition processes have concen-

trated on microscopic and microbiological examination of peats in order to gain some indication of the nature and rate of the decay process. Dickinson, Wallace and Given (*New Phytol.*, **73**, 107; 1974) have examined peats from the Florida Everglades and used three different methods for determining microbiological activity at different depths. They examined and counted the microorganisms present; they washed, plated and incubated peat samples; finally, they incubated samples from freshly exposed peat surfaces. Direct counts showed large numbers of microorganisms at all depths, but it is possible that the bulk of these were dead. The other two techniques showed a strong concentration of viable organisms within the surface layers of peat, as Clymo's work had suggested for *Sphagnum* peats. Once again, the inference is that the bulk of decomposition occurs in the superficial peat horizons.

An alternative approach has been used by Dickinson and Maggs (*New Phytol.*, **73**, 1249; 1974), who observed the results of decomposition on *Sphagnum* leaves by both light microscopy and scanning electron microscopy (see figure). They devised scores for different degrees of degradation and made estimates of the proportion of *Sphagnum* leaves in each condition at various depths down to 30 cm. Decay was considered to be due to the activity of microflora rather than fauna. Readings were taken at only four levels, making it difficult to discern any precise pattern of degradation with depth; however it would seem that about 20–30% of *Sphagnum* leaves had undergone some degradation at 16 cm depth and about 30–40% at 30 cm depth. Without information about accumulation rates of peat during the time that this 30 cm of peat had built up, it is impossible to infer from these data the precise pattern of decay processes. If, for example, peat accumulation has been fast in the past but is now slowing down it would be possible for material from deep in the peat to be better preserved than that near the surface.

Ultimately the method of Clymo and

Reddaway (*Moor House Occasional Papers*, No. 3; 1971) is likely to prove the most helpful for production ecologists concerned with peatland decay rates. They collected gaseous output from a peatland surface and assessed the flux of carbon dioxide and methane; they also measured organic losses in solution. The gaseous measurements can be considered as the respiratory products of producer, consumer and decomposer organisms from a complete vertical column of the peatland system. Their figures indicate that overall dry matter surplus (available for peat formation) in a Pennine blanket bog varies between $1 \text{ g dm}^{-2} \text{ yr}^{-1}$ on hummocks to $2.7 \text{ g dm}^{-2} \text{ yr}^{-1}$ on *Sphagnum* lawns. In spite of difficulties in measurement and the high variability of data with surface flora and microtopography, this still represents the most promising approach to the measurement of the respiration and potential growth rates of bogs.

Total reconstitution of *E. coli* 50S ribosomes

from a Correspondent

RECONSTITUTION of the *Escherichia coli* 30S ribosome from its constituent RNA and protein has been well established for several years (Traub and Nomura, *Proc. natn. Acad. Sci. U.S.A.*, **59**, 777; 1968), but total reconstitution of the corresponding 50S particle has proved a much tougher nut to crack. A reassembly procedure was described by Maruta, Tsuchiya and Mizuno (*J. molec. Biol.*, **61**, 123; 1971) using proteins liberated by the action of ribonuclease II, but so far no report has appeared of a successful repetition of this result. 50S ribosomes from *Bacillus stearothermophilus* have been reconstituted (Nomura and Erdmann, *Nature*, **228**, 744; 1970) by taking advantage of the higher thermal stability of ribosomes from this organism, but this of course is not so useful since most of the biochemical and genetic information concerning ribosomes has been accumulated from *E. coli*. Hitherto, biochemists have had to content themselves with the 'partial reconstitution' method for *E. coli* 50S ribosomes. This was first reported simultaneously by Staehelin and Meselson (*J. molec. Biol.*, **15**, 245; 1966) and by Hosokawa, Fujimura and Nomura (*Proc. natn. Acad. Sci. U.S.A.*, **55**, 198; 1966) who both showed that protein-deficient 'core' particles could be reassembled to active 50S ribosomes. Now, however, the total reconstitution of *E. coli* 50S particles from protein and RNA has been achieved, by a two-step incubation procedure, and is reported by Nierhaus and

Dohme in *Proc. natn. Acad. Sci. U.S.A.* (**71**, 4713; 1974).

Nierhaus and Dohme began by finding out how many proteins could be removed from the 50S particle before the resulting core particle became unable to regain its activity in the partial reconstitution procedure. Activity was measured both by the poly(U) assay, and by the 'fragment assay' for polypeptidyl transferase. They found that whereas 4 M LiCl cores could still regain activity, 5 M cores (containing only five proteins) had largely lost this ability, and 6 M cores (with only two proteins) could not be reconstituted at all. The obvious next step—a stepwise reassembly of 23S RNA to a 4 M core particle, and thence to a 50S ribosome—was not successful as judged by the fragment assay, although some activity in the poly(U) assay was recovered. Nierhaus and Dohme rightly interpreted this failure as an indication that the ionic conditions of the partial reconstitution procedure were not ideal for the early stages of total reconstitution.

They therefore mixed 23S RNA, 5S RNA and total protein, and investigated the effect of various incubations prior to the normal partial reconstitution. By optimising the conditions they arrived at a two-step procedure in which the isolated ribosomal components were first incubated together for 20 minutes at 40°C in 4 mM magnesium, 400 mM ammonium at pH 7.2, followed by the partial reconstitution incubation of 90 minutes at 50°C in 20 mM magnesium, 400 mM ammonium. This gave particles with a remarkably high activity, that is to say 80–110% in the poly(U) assay, and 40–60% in the fragment assay, as compared with native 50S sub-particles. Notably, no requirement for 30S particles or polyamines in the reconstitution mixture was observed. Phenol-extracted RNA and acetic acid-extracted proteins were used in the procedure, in order to ensure that the RNA fraction was entirely free from protein and the protein fraction entirely free from 5S RNA. This can therefore be regarded as a genuine total reconstitution, and should prove as advantageous to the understanding of the 50S particles as the corresponding reconstitution has already proved to the 30S. Detailed 'assembly mapping' of the 50S particle should now follow without too much difficulty.

Pituitary neuropeptides and behavioural processes

from A. Dickinson

THE idea that pituitary neuropeptides can influence behaviour by a direct action on the central nervous system is

a recent one. These effects of pituitary hormones seem to be distinct from their well known actions on target tissues such as kidney and adrenal cortex; they resemble rather the actions on the CNS of steroid hormones secreted by endocrine target tissues.

The relation of pituitary neuropeptides to learning processes was one of the topics discussed at the fourth winter school of the European Training Programme in Brain and Behaviour research (January 4–11). Some of the strongest evidence for a direct action on the CNS comes from the work of the Utrecht group on the effects of adrenocorticotrophic hormone (ACTH) and related peptides on avoidance behaviour. In their experiments an animal is required either to make some response (active avoidance) or to refrain from responding (passive avoidance) in order to avoid an electric shock. They showed previously that injections of ACTH restored the deficit in active avoidance induced by hypophysectomy and prolonged active avoidance in intact animals during extinction. ACTH also improves passive avoidance acquisition. The important finding is that these behavioural effects seem to be independent of the adrenocorticotrophic activity of ACTH since the injection of ACTH 4–10, a fragment of the ACTH peptide chain which has no adrenocortical stimulating activity, produces comparable changes in avoidance behaviour.

Dr B. Bohus's report on this work made it clear that the facilitatory influence of ACTH was only seen while the hormone was present in the body. It was therefore suggested that the primary central effect of ACTH is to increase the general arousal or motivational state of the animal. Such a general effect is in line with biochemical studies reported by Dr W. H. Gispen (Institute of Molecular Biology, Utrecht) showing that the behaviourally effective fragments of ACTH seem to increase the incorporation of labelled leucine into almost all brain stem proteins of the rat.

Dr J. A. Gray of the University of Oxford reported a complementary series of experiments using learned appetitive responses rather than avoidance responses. After training approach responses by positively reinforcing them with rewards such as food and water he extinguished the responses by withdrawing the reward and found that ACTH 4–10 prolonged such extinction. Though at first sight his results seem to be comparable to those of the Utrecht group, it is not clear that the interpretation given above for the Utrecht results will explain those of Dr Gray. Withdrawal of a reward engenders emotional arousal such as frustration; such aversive arousal actually

helps to produce extinction and an ACTH-induced increment in arousal might therefore be expected to shorten extinction of a positively reinforced response while prolonging the extinction of avoidance behaviour.

Dr Gray has provided further evidence that ACTH attenuates the emotional responsiveness of an animal as measured by positively reinforced responses. Tranquillisers such as sodium amylobarbitone seem to produce the same behavioural effects during the extinction of these responses as exogenous ACTH. Furthermore, Dr Gray has shown that the ACTH 4-10 fragment increases the threshold for driving a specific electrical rhythm of the hippocampus (the theta rhythm) from the septum in the same manner as sodium amylobarbitone. Previous Oxford work has implicated this system in the mediation of the behavioural effects of nonreward.

A second pituitary hormone with interesting behavioural effects is vasopressin. The Utrecht group has shown that this neuropeptide and its analogues without antidiuretic or pressor action have effects similar to those of ACTH on avoidance behaviour. They differ from ACTH in having an influence which lasts longer than their presence in the body. Dr Bohus suggested that vasopressin influences memory processes by affecting the consolidation or retrieval of learned responses. Using a passive avoidance task in which rats learned to suppress a response in one trial, he reported that animals with a lack of vasopressin through hereditary hypothalamic diabetes insipidus showed good retention if tested immediately after training but not if testing was delayed for three or twenty-four hours. If registration and storage-retrieval processes really can be distinguished using this neuropeptide, it may provide a powerful tool in the analysis of learning and memory.

In the study of the pituitary neuropeptides, psychological theory meets fruitfully with the other neurosciences. The effects of exogenous ACTH on the performance of learned tasks, effects which do not fit easily into the current behavioural analyses of these tasks, provide an important guide for revising both behavioural theories and concepts about the sites and modes of action of neuropeptides in the CNS.

Chilling statistics on Cyprus

from Peter J. Smith

THE sheeted diabase unit of the Troodos complex in Cyprus is a complicated geological feature whose stratigraphically central section is formed completely of dykes intruding other

dykes. The unit as a whole has an exposure in the east-west direction of more than 70 km, although the individual dykes, which generally strike north-south, range in width from a few metres to only a few centimetres. The periods between dyke injections were apparently sufficient to allow the previous dykes to cool, so that each new dyke was chilled against those into which it was intruded and thus has finer-grained or cryptocrystalline margins. But as many of the dykes are intruded by later ones, they may have been split several times, forming apparently marginless dykes which are in reality merely the central parts of once complete intrusions. And if that is not enough to cause confusion, some dykes intruded up the margins of previous dykes, so that the later dykes were chilled against the chilled margins of the earlier ones.

What is the origin of this remarkable structure? The answer to that question would be interesting enough if the diabase unit were only of purely local significance. But the Troodos complex is, of course, an ophiolite suite comprising ultramafics, gabbro, sheeted dykes, pillow lavas and deep sea sediments—a feature widely believed to represent ancient ocean floor. If the origin of the diabase unit could be determined, the result would give by implication the origin of the whole interrelated complex; and geologists would be sure at last whether or not they could claim access to ocean floor without the need for deep ocean diving.

The answer most geologists would welcome would be proof that the diabase dykes were injected at, or close to, an oceanic spreading axis. But as Kidd and Cann (*Earth planet. Sci. Lett.*, **24**, 151; 1974) point out, a structure composed entirely of dykes could be produced in two other ways—by random injection throughout the whole structure (that is, with a zone of intrusion much wider than that implied by seafloor spreading) or by the coalescence of dyke swarms from individual volcanoes.

Fortunately, however, the three models do not predict the same detailed dyke swarm structure. Consider the case of ideal seafloor spreading, in which the width of the zone of intrusion is infinitely small and in which each new dyke intrudes up the centre of the preceding one. On either side of the intrusion zone the oceanic crust will comprise entirely half-dykes, each having a chilled margin on the side away from the intrusion zone. In other words, on each side of the intrusion zone the magnetic vectors within the chilled margins will all lie in the same direction (they will be all 'one way') and the degree of 'one way chilling' will be 100%.



A hundred years ago

THE *Kölnische Zeitung* of Feb. 7 contains an abstract of a paper read by M. G. Wex, at the Geographical Society of Vienna, on the decrease of water in rivers and sources. The author states that the results of his observations tend to show the constant decrease of the rivers of Germany and the increase of seas. It appears from them that the levels of the German rivers are now much lower than they were fifty years ago; viz., the Elbe 17 in., the Rhine 24.8 in., the Oder 17 in., the Vistula 26 in., the Danube 55 in. As a reason for this decrease, the author gives the progressing devastation of forests, which causes a decrease in the atmospheric moisture they attract and convey to the soil and thence to sources. from *Nature*, **11**, 314; February 18, 1875

In any real spreading system, complete 'one-way chilling' will never be achieved because the intrusion zone will have a finite width and the splitting of previous dykes will not be as regular as in the ideal case. Nevertheless, as long as the intrusion zone is not too wide, some of the dykes will have been intruded close enough to the centre of injection for them to have been split by subsequent injections; and in such a case the degree of one way chilling, though far from 100%, should be significantly greater than zero. It is this statistical point which should enable the seafloor spreading model to be differentiated from the other two. Where intrusion takes place throughout the whole dyke region or where it is derived from several discrete volcanic centres, there is no single effective centre of injection and thus presumably no bias in the chilled margin directions.

Kidd and Cann have now applied these ideas by taking a number of well exposed and widely scattered sections within the Troodos dyke unit and measuring the directions of chilling of an odd number of margins within each section. Because the number of chilled margins within each section is always odd, there is bound to be an excess of margins in one direction or the other (east or west in this case because the margins trend north-south). But whereas the seafloor spreading model would predict a significantly greater number of sections with an excess in one direction rather than the other (the one direction being that away from the spreading centre), the two other models would predict no such imbalance.

In practice, the statistical bias is confirmed. Out of 23 section transects covering 41 chilled margins each, 19 transects (TRE) showed a preponderance of margins chilled to the east over margins chilled to the west and 4 transects (TRW) showed a preponderance the opposite way. The chance of obtaining such a bias from a system which actually contains equal numbers of TREs and TRWs is less than 1 in 200. This suggests that the statistics reflect a real physical effect, namely, that the Troodos dyke unit was produced as part of the seafloor spreading process. This conclusion is further supported by the number of chilled margins actually in excess within each transect. For transects with margins predominantly to the east the average number of margins in excess within each transect is 2-3, whereas for transects with margins predominantly to the west the corresponding figure is only 1.

Finally, since the predominant chilled margin direction is the direction away from the spreading centre, it follows from the data above that the spreading axis should have lain relatively to the west of the Troodos complex. It is necessary to say 'relatively' here because Moores and Vine (*Phil. Trans. R. Soc., A268*, 443; 1971) concluded from palaeomagnetic evidence that the complex has rotated through 90° in an anti-clockwise direction since it was formed, which implies that the dykes within the diabase unit were originally intruded in an east-west direction and that any spreading axis would have lain to the north. But although the orientation of the supposed spreading system is important, it is far more important for the time being to show that spreading took place at all. The simple, but elegant, piece of work carried out by Kidd and Cann provides further evidence that it did.

Copernicus and X-ray astronomy

from A. C. Fabian

THE Copernicus satellite launched in 1972 carries an 80 cm ultraviolet telescope-spectrometer from the Princeton University Observatory measuring interstellar absorption lines superimposed on stellar spectra. The small companion X-ray package from the Mullard Space Science Laboratory at University College, London, contains three grazing-incidence telescopes and a collimated proportional counter of about 15 cm² aperture. Both sets of instruments are pointed to the same location in the sky to fractional arc second accuracy by the inertial guidance system. Thus the ultraviolet telescope sometimes observes X-ray sources, and the X-ray systems spend

some of the time viewing hot stars.

In spite of this apparent clash of interest, much of the work with the X-ray package has depended upon the Princeton telescope. Fifty per cent of the light incident on the Princeton spectrometer is used in a fine error sensor to provide the primary pointing control for the satellite and a stellar reference system for the X-ray experiment. The resultant accurate pointing has allowed maps of supernova remnants and clusters of galaxies to be made. Scans of a number of X-ray sources in our Galaxy and the Large Magellanic Clouds have enabled their positions to be pinpointed within a few arc minutes. Identification with optical, radio or infrared counterparts is proceeding.

The failure of two of the X-ray telescopes in July 1973, owing to a jammed shutter blocking the light paths, has caused mapping operations to cease. Nevertheless, the collimated proportional counter which operates over a 2.5-10 keV range has not been idle. It is well known that the binary X-ray sources are highly time variable. The stable platform provided by Copernicus has allowed careful studies to be made of some of these X-ray binaries. The data so obtained are superior to those from scanning instruments such as *Uhuru* on time scales exceeding several minutes, owing to this accurate pointing. The larger area X-ray detectors on the recently launched Ariel-5 that view along the spin axis also enjoy some pointing stability, but drift of the spin axis will introduce uncertainties.

Source identifications can still be made through correlated X-ray and optical or infrared observation. Lunar occultations also provide a powerful technique, and have been successfully applied from Copernicus to a galactic centre source, GX5-1 and the Crab nebula. The motion of the satellite allows several passages of the Moon across these sources to be observed.

Many more X-ray observations have been proposed for 1975. At least one complete 'on'-state of the 35-d cycle of Hercules X-1 will be followed. This enigmatic binary system has been intensively studied optically over the past two years but the mechanism behind its 35-d cycle is still unclear; complete and accurate X-ray data are lacking. Several other binaries and suspected binaries will be monitored for extended periods. The tight schedules of the other X-ray detectors in orbit do not permit extended study of particular objects because of the need to conserve stabiliser gas in the case of UK-5 and the viewing constraints for the ANS. It is hoped that the X-ray observing programme of Copernicus will continue, for there is no lack of sources wanting investigation.

Nuclear spectroscopy

from P. E. Hodgson

STUDIES of nucleon transfer reactions have long been established as one of the most powerful nuclear spectroscopic techniques. The angular distribution is characteristic of the angular momentum transfer L and the magnitude of the cross section is a measure of its single-particle strength. If the target nucleus has spin zero and the transferred particle is initially in a state of zero orbital angular momentum the spin of the final state is simply given by the vectorial sum of the relative orbital and spin angular momenta in the transfer process. For a (d,p) reaction, for example, $J=L+\frac{1}{2}$ giving $J=L+\frac{1}{2}$. Thus a $L=2$ transfer can go to a $J=3/2$ or to a $J=5/2$ state. The ambiguity in sign can often be resolved from the known systematic behaviour of single-particle states but in other cases it remains a difficulty. Measurement of the polarisation of the outgoing proton together with distorted wave calculations or a simple comparison with the proton polarisations in reactions leaving the residual nucleus in states of known spin resolves the ambiguity, but is time consuming.

It has however proved possible in some cases to resolve the ambiguity by examination of certain features of the differential cross sections and it is found that they are characteristic of $J=L+\frac{1}{2}$ or $J=L-\frac{1}{2}$ final states. Such features are sometimes difficult to reproduce by distorted wave calculations but nevertheless can be used as empirical guides provided that they can be calibrated by studies of transitions to states of known spin.

These J -dependent effects, as they are called, are sufficiently distinctive to be used with confidence only for particular reactions in restricted energy ranges, so it is always valuable to find new circumstances or new reactions that display them prominently. Two recent papers have provided evidence of this type.

In the first paper, Kong-a-Siou and Chien of Michigan State University (*Phys. Lett.*, **52B**, 175; 1974) measured the angular distributions of several $L=3$ transitions in the (p,d) reaction on ⁶¹Ni and ⁶²Ni at 40 MeV and found a very stable J dependence over a range of intensities and excitation energies. Some of their results are shown in Fig. 1, and it is clear that the angular distributions fall sharply into two classes, one characteristic of $J=L-\frac{1}{2}=5/2$ and the other of $J=L+\frac{1}{2}=7/2$, and that in each case the data fall quite definitely on one or the other. The data for ⁶²Ni(p,d)⁶¹Ni thus allow the spins of the states of ⁶¹Ni to be determined.

The results for ⁶¹Ni(p,d)⁶⁰Ni to states

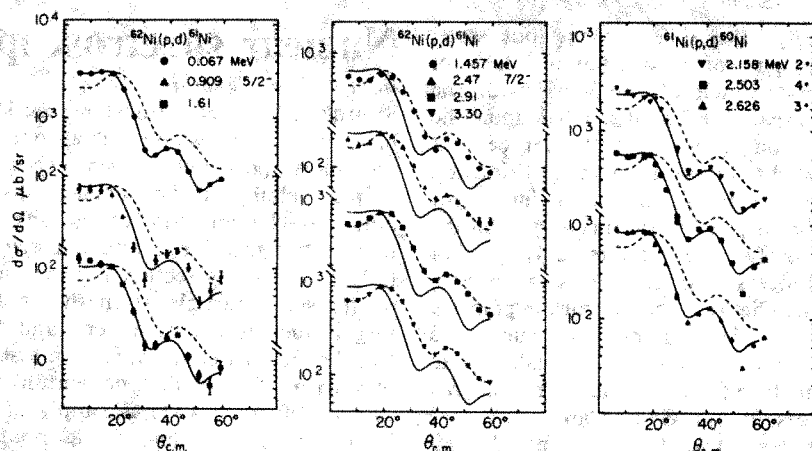


Fig. 1 Differential cross sections for the $^{61}\text{Ni}(p, d)^{60}\text{Ni}$ and $^{62}\text{Ni}(p, d)^{61}\text{Ni}$ reactions. The solid and dashed lines are smooth curves through the experimental data points of the known $f_{5/2}$ and $f_{7/2}$ transfers to the states at 0.067 and 3.30 MeV in ^{60}Ni . All the cross sections follow closely one curve or the other.

of known spins 2^+ , 4^+ and 3^+ respectively show angular distributions characteristic in each case of quite pure $J=5/2$ transfer. For the 4^+ state, only $L=3$ transfer is allowed by the conservation of angular momentum and of parity, but for the 2^+ and 3^+ states both $L=1$ and $L=3$ transfers can complete. The data show that only $L=3$ is present and this can indeed be understood by shell-model calculations.

The second paper, Kemper *et al.* of Florida State University (*Phys. Lett.*, **52B**, 179; 1974) report a study of the relatively unfamiliar (^7Li , ^6He) reaction that shows marked J -dependent effects for reactions to d and f states. For this reaction the transferred proton comes from the $p_{3/2}$ orbit in ^7Li so that the vector equation for the angular momenta is $3/2 + l = J$, where l is the orbital angular momentum of the transferred proton and J the spin of the final state. If for example $J=3/2$, then l can be 0, 1, 2 or 3. If the final state has orbital angular momentum L , then J can be $L \pm 1/2$ and the possible values of l are $l=L$ or $L \pm 1$. The relative proportions of these that contribute are given by the Racah coefficient

$W(L, J, 1, 3/2; 1/2)$ and it turns out that the components $l=L$ and $l=L-1$ are much more important for the states with $J=L+1/2$ than for those with $J=L-1/2$.

This is shown clearly in Fig. 2 by the angular distribution for the $^{24}\text{Mg}(^7\text{Li}, ^6\text{He})^{23}\text{Al}$ reactions at 34 MeV to $d_{3/2}$ and $d_{5/2}$ states of ^{23}Al . The curves show separately the distorted wave calculations of the contributions to the two cross sections, and it is found that the reaction to the $J=3/2$ state ($J=L-1/2$) is nearly all $l=3$ with very small contributions from $l=1$ and 2, whereas for the reaction to the $J=5/2$ state the $l=1$ and 2 components are relatively much more important. Since the contributions of different l have markedly different angular distributions this gives a difference between the cross sections for the reactions to the $J=3/2$ and $J=5/2$ states, and this can be used to distinguish between them.

Reactions like this are only just beginning to be studied in detail, and it is likely that other types of J dependence will be found and that they will prove powerful tools in nuclear spectroscopy.

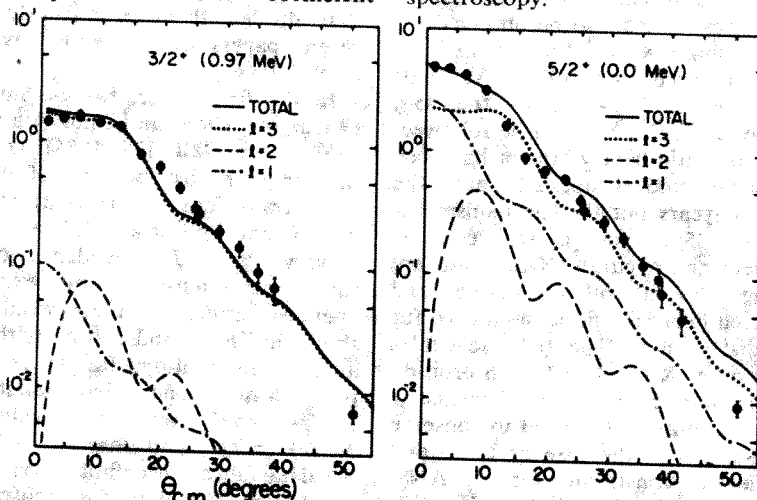


Fig. 2 Differential cross sections for the $^{24}\text{Mg}(^7\text{Li}, ^6\text{He})^{23}\text{Al}$ reaction to $d_{3/2}$ and $d_{5/2}$ states in ^{23}Al . The curves are distorted wave calculations and show the difference between the relative contributions from $l=1, 2$ and 3 transfer in the two cases.

Palaeoscience and the modern world

from J. A. Taylor

WHAT relevance have the palaeosciences to the interpretation and solution of the environmental problems of the modern world? Judging by the meagre support given to some of the palaeosciences (notably palaeobotany, palaeopedology and palaeoclimatology) by the Natural Environment Research Council, the answer would be "precious little". Yet the improved techniques of the palaeosciences are finding uses in present-day ecological studies, thus bridging the gap between the study of ancient and modern environments. At a symposium of the Biogeography Study Group held in Oxford on January 1, methods were described for absolute dating, and for dating very recent objects. Palaeomagnetic dating and lead and caesium analysis are now becoming available to supplement pollen records and radiocarbon assays already corrected for errors detected by dendrochronological research. Again, in the field, magnetic susceptibility measurements are proving an invaluable aid in selecting representative single cores from extensive peat bogs, lake basins or peaty moorlands.

F. Oldfield (University of Lancaster) reported the preliminary but successful application of these new techniques in environments as contrasted as Sweden and New Guinea. His advocacy of the lacustrine sediments as a superior source of continuous and complete palaeochronologies is, however, open to some criticism. Such sediments may incorporate successively younger layers, duplicated or polycyclic layers, unconformities due to erosion, and homogenisation, due to alluvial and colluvial mixing, as B. Seddon (University of Reading) reported for his detailed pollen record for sediments at Llangorse lake in Powys, Wales. Clearly, differentiation between lacustrine and terrestrial sources and between riverborne and airborne pollen is necessary. It is thus desirable to examine modern pollen cycling within the adopted lake catchment and to analyse soil pollen records and pollen evidence from peats and muds.

Several papers described local palynological studies which have regional significance in Britain. It is beginning to be possible to reconstruct vegetation cover for different culture periods. Some changes, such as elm decline, are broadly synchronous (though substantial variations in timing occur at certain Welsh sites); others, such as man-made clearances between Mesolithic times and the Iron Age are more diachronous. It is becoming clear that late Mesolithic man was considerably

more advanced than archaeologists have previously believed. He partially domesticated the red deer and initiated sizeable forest clearances.

In comparing old, middle-age and young soils on Exmoor K. Crabtree (University of Bristol) and E. Maltby (University of Exeter) estimated the developmental stage reached by a soil profile by measuring the rate of accumulation of organic matter over time. By this technique again a process can be calibrated from its inception to the present day, eventually absolute soil chronologies should be available which will allow refined measurement of the ecological stability and trend of individual soil environments as related to management and planning.

The problems which recur when attempts are made to interpret archaeological evidence in environmental terms were well exposed by D. A. Davidson (University College, Lampeter) and R. L. Jones (Lancaster Polytechnic) who examined the location and distribution of Neolithic chambered cairns in the Orkneys. A model for the island of Rousay evaluated seven environmental parameters of cairns: distance from coast, nature of coast, slope, soil drainage, rockiness, altitude and visibility from the mainland of Orkney. The sites generated were compared with the actual distribution of cairns and the best approximations were found to occur for (1) nearness to a beach coastline and (2) steepness of slope. W. Kirk (Queen's University, Belfast) criticised any rationalisation of the location of cairns since they were burial places and not settlement sites. But as Frances Lynch (University College of North Wales, Bangor) has argued (in *The Effect of Man on the Landscape Highland Zone*, Council for British Archaeology Research Report II, in the press), prehistoric man may have been discriminating in the selection of both vantage points and viewpoints for burial as well as settlement. A subtle blend of the defensive and the exhibitionist, and of the imitation of natural features, may sometimes be inferred.

Lunar eclipses and Danjon's law

from David W. Hughes

LUNAR eclipses occur when the Earth's shadow is cast over the full Moon. They have an average duration of 226 min, totality (full shadow) lasting about 103 min. Sixteen lunar eclipses occurred between 1965 and 1975, the exact time of occurrence depends on the relationship between the draconic (lunar nodal) month, the synodic (identical phase) month, the precession of the nodes of

the orbit and the Earth-Moon-Sun geometry. In totality the Moon presents a fascinating spectacle—the contours of the seas and some of the craters are discernible, its hue is distinctly red and its brightness and colour vary from limb to centre creating the impression of a sphere as opposed to the flat disk of the normal full Moon.

The reddish glow is an interesting phenomenon. Rays of sunlight just grazing the edge of the Earth's globe must pass right through the thickest part of the atmosphere where they undergo refraction and are bent into the cone of shadow and fall upon the eclipsed Moon. The light also undergoes absorption due to Rayleigh scattering, the short wavelengths are attenuated much more than the long, leaving the remaining light red like sunsets on Earth. The spectrum of the light reflected from the eclipsed Moon and its variation across the disk can be explained by the absorption of light in the terrestrial ozone layer and can be used to give a concentration-height profile of ozone in the Earth's atmosphere.

In 1920 Danjon, a French astronomer, noticed a relationship between the phase of solar activity and the brightness of a lunar eclipse (*C. r. Acad. Sci. Paris*, **171**, 1127, 1920). This relationship is now known as Danjon's law and can be stated as follows: in the two years immediately after a solar activity minimum the shadow of the Earth during a lunar eclipse is very dark and has little colour. As the solar activity moves away from minimum the eclipsed Moon becomes brighter and redder until, during the seventh and eighth year after solar minimum the eclipsed Moon is at its brightest and is red, copper coloured or orange. The brightness curve then falls away very sharply to its minimum value. The maximum phase of the solar cycle passes unnoticed whereas the minimum phase is indicated by a sudden and considerable diminution in brightness and colour, this change forming a discontinuity.

To obtain this law Danjon had analysed eclipse records dating back to the time of Tycho Brahe. He subsequently used the law to estimate the times of solar minimum during the 17th, 18th, and 19th centuries, finding the general relationship that minima occurred in the years $1584.8 + 10.87n$ where n is an integer (*C. r. Acad. Sci. Paris*, **171**, 1207, 1920). Notice that the length of the solar cycle, 10.87 years as derived from lunar eclipses, is less than the currently used value of 11.2 years derived from sun spots.

Link, of the Institut d'Astrophysique, Paris, has reconsidered the underlying causes of the variations of lunar eclipse brightness described by

Danjon's Law in a recent edition of *The Moon* (**11**, 137, 1974). He finds that the phenomenon behind the law is not the absorption of the light in the terrestrial atmosphere, as originally assumed by Danjon, but some light source other than the normal refracted solar illumination. The problem is to find this source. Vassy (*J. Sci. Mét.* **8**, 1, 1958) hypothesised that the cause is a scattering layer in the Earth's upper atmosphere produced by aerosols. The spatial density of these aerosols could be affected by the corpuscular radiation flux from the Sun and the variation in density caused by the sudden change in the heliographic latitudes of the source of this radiation after solar minimum. Link examines the possible implication of this hypothesis on the twilight and daytime sky as observed from Earth. Looking at two extremes of shadow density he finds the eclipse of January 18, 1954 to be a thousand times less luminous than that of September 26, 1950. To explain the bright eclipse by aerosol scattering requires an aerosol density such that the twilight brightness would be a hundred times more than observed. The luminance of the daylight sky too is far from that needed to explain Danjon's Law.

Another source of additional light is the luminescence of lunar surface materials. This could be caused by corpuscular radiation striking the Moon during the eclipse or it could be an afterglow from the period preceding the eclipse. But recent laboratory examinations of lunar samples showed that the luminescence is much too low to explain the eclipse observations.

Two other facts must be borne in mind: first Fisher (*Smithson Misc. Coll.*, **76**, No 9, 1924) found winter eclipses to be brighter than those occurring at other seasons and second, exceptions to Danjon's Law have occurred when very dark eclipses were seen after the eruption of the volcanoes Krakatoa (1883) and Katmai, Alaska (1913).

But we must return to Link's final conclusions, that a scattering layer in the upper atmosphere, or luminescence of the lunar surface—both caused by solar corpuscular radiation—describe qualitatively the general features of Danjon's law but are quantitatively completely unsatisfactory. So the cause of the relationship remains unknown.

Erratum

In the report "G-wizardry at Dallas" by John Faulkner (**253**, 231, 1975) the scalar coupling constant was printed in the penultimate paragraph as $\gtrsim 23$ instead of $\lesssim 23$. Also Colgate's institution is New Mexico Institute of Mining and Technology, not University of New Mexico as printed.

THE central theme of the symposium was structure at the molecular level. Many fibrous proteins have a highly regular structure, for example, the collagen of tendon shows nature adopting at the molecular level the principle of successive twisting that man has developed in the rope. Man has not yet developed a molecular rope, but different methods of drawing polyethylene lead to fibres with a wide variety of mechanical properties, some with the stiffness of glass and the tensile strength of steel (Ward, Leeds). The mechanical properties may be related to the structural regularity of the molecular folds in the polymer. There is a striking similarity between the folded chains of polyethylene (Sadler, Bristol) and those of the protein in the egg stalk of the lacewing fly, *chrysopa*, but many natural polymers share with the synthetics an intermingling of regular and less regular structural regions. In the globular enzymes, the same types of chain conformation are found as in fibrous proteins, but each stretch is limited in extent so that the chain can fold to form a compact shape (North, Leeds). The recurrence of certain well defined folds in different enzymes probably indicates evolution from a common ancestral protein, though there remains the possibility that some conformations may recur just because they are energetically favourable, as do the folds in polyethylene.

The alternation of regularity with irregularity is seen too in polysaccharides, the formation of gels of which depends upon molecular chains being associated in pairs for limited distances (Rees, Unilever), interruption of the regular alternating copolymer by a sugar unit with a different conformation makes a kink in the chain, causing the molecular partnership to break and the two chains to diverge before joining up with different partners. The variety of sugar units utilised in polysaccharides permits a great range of conformations, some of which are very susceptible to changes in solvent environment (Atkins, Bristol), thus conformational changes similar to those induced in heparan sulphate by calcium may be a vital factor in cell adhesion.

In the chromatin of the cell nucleus, the nucleic acid is intimately associated with histones, basic proteins of five types. It is now beginning to appear that the histone molecules are individually globular and associate specifically to form a bead-necklace around which the nucleic acid is wound (Thomas, Cambridge).

A second theme of the conference was chemical modification of polymers. Technologists may attempt to modify a polymer in order to improve its properties, such as the drape and

Natural and synthetic polymers

With the praiseworthy intent of allowing enzymologists and polymer scientists to learn from each other, the SRC Enzyme Chemistry and Technology Committee organised a meeting on January 2 and 3 at Cambridge. In three sessions the meeting dealt with synthetic polymers, structural natural polymers and enzymes. The reports of the meeting which follow describe it from the point of view of, respectively, an enzymologist and a polymer scientist

crease-resistance of cotton fabrics (Roberts, Shirley Institute). Protein workers often use chemical modification to a different end, in probing structure and function. Very high degrees of specificity may be achieved (Dixon, Cambridge), though care must be exercised in drawing conclusions from the effects of such modifications—the activity of an enzyme may be perturbed by changes remote from its active site. Such methods have been applied elegantly in distinguishing the part played by individual subunits in a multimeric enzyme and in studying the interactions between components of a multi-enzyme complex and between the subunits of a virus (Perham, Cambridge).

The Cambridge meeting established interesting overlaps between the work on natural and synthetic fibres, for example in the molecular basis of mechanical properties and in the thermodynamic and kinetic constraints on the folding pathways. While the programme had a bias towards the natural fibres, showing clearly the great subtlety, elegance and efficiency with which nature has evolved a wide variety of functions from a limited repertoire of structural elements, it is clear that man has already achieved enormous progress in a very short time in making an almost comparable variety of synthetic materials.

Experiments with model compounds (Kirby, Cambridge) show how chemical reaction rates depend critically upon the reacting groups being constrained into an appropriate geometry, such studies are vital to the eagerly awaited development of catalysts with the efficiency and specificity of enzymes—which will doubtless owe much to comparative studies of natural and synthetic polymers.

A C T NORTH

SCIENTISTS have great trouble even in communicating the meaning of their work to each other. This was probably the main conclusion to be drawn from the symposium which, because of the lack of dialogue or (apparently) of flashes of inspiration, must be judged a failure. The reasons may be that the speakers did not address themselves sufficiently to areas of potential overlap, that the discussion was too limited or that the two subjects are irrelevant to each other.

Polymer science groups in the USA are increasingly turning their interest to biological macromolecules, spurred on by the increase in the fortunes of the NIH compared to those of the Defense Department a few years ago. This has resulted in work on solid state and solution properties of polysaccharides and polypeptides, one obvious avenue, and in the study of collagen ultrastructure and its relationship to mechanical properties presented by R G C Arridge and L Gathercole of Bristol and carried out jointly with Case Western Reserve University.

One thing that did emerge was the relative lack of understanding of physical aspects of enzyme structure such as the denaturation and renaturation processes, the nature of subunit interactions, and of questions such as "How large must a protein be to have a hydrophobic core?" posed by T L Blundell during the discussion. This was reinforced by the parallel between J O Thomas's review of work on chromatin structure at Cambridge and D M Sadler's of polymer crystallisation and morphology. Both topics are almost baroque in their complexity, the methods used are completely different, yet the problems are very much related. It is to be hoped that researchers starting from the synthetic polymer direction can contribute to these subjects, though the immediate problem for a person used to working with kilograms of sample is to learn techniques for handling natural polymers, and here cooperation between the groups is essential.

P D CALVERT

articles

The two geological time scales

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Several international bodies are currently preoccupied with the task of establishing, defining and standardising time scales for geology. Geological ages may be expressed on either of two alternative scales often distinguished somewhat misleadingly as 'relative' and 'absolute' ages

THE chronostratigraphic scale (with its hierarchy of eon, era, period, epoch, age, and chron) has developed slowly over more than 150 years. Its divisions were initially unconformities, conceived of as natural chapters in Earth history, but these were later superseded by divisions defined palaeontologically. The scale is currently being redefined on a global basis, with boundaries standardised at reference points in type sections, in order to produce a Standard Stratigraphic Scale¹ or Standard Global Chronostratigraphic (Geochronologic) Scale². The principle has been illustrated by the establishment of one point on the scale—the Silurian–Devonian boundary³.

The geochronometric scale⁴, unlike the chronostratigraphic scale is based on a unit of duration, originally defined as the geochrone⁵, but since the development of radiometric methods the terrestrial year has been the only unit in general use. The geochronometric scale is periodic, that is, simply the numerical multiplication in steps of 1,000 of the agreed unit ($10^0\text{yr} = 1\text{a}$, $10^3\text{yr} = 1\text{ka}$, $10^6\text{yr} = 1\text{Ma}$, $10^9\text{yr} = 1\text{Ga}$). This scale is already in use whenever an age is expressed in years. The geological year is not yet standardised as it is necessary to choose between the Ephemeris 1900 year of the International Union of Astronomers 1957, and the year based on the caesium second of the International Conference of Weights and Measures 1957, but that is of theoretical interest only¹. Of practical interest, however, is the often expressed wish to divide the whole scale into longer spans of time with internationally agreed names.

The terminology used for the two scales is in some doubt for many years the term 'geochronology' has been used mainly within the context of the geochronometric scale (for example, IUGS Subcommittee of Geochronology), whereas the IUGS Subcommittee of Stratigraphic Classification (following the American Stratigraphic Code) has used the same term when referring to time spans of the chronostratigraphic scale². To avoid confusion the term 'geochronometric' can be used unambiguously for a scale based on years as the term 'geochronology' might become used generally with both scales.

Both are framework scales⁶, they both express time but in quite different ways. They are both artificial, they cannot be discovered but only decided by convention (for example, 'golden spikes' for the chronostratigraphic scale, and a standard second for the geochronometric scale). The framework allows the expression of the ages of phenomena in a common language and the phenomena (biological evolution, magnetic reversals, climatic fluctuations, tectonic events, nuclear decay, and so on) are necessary for correlation and the 'determination' of age. It is an historical consequence and not a scientific necessity that previously has led biostratigraphy to be concerned largely

with the development of the chronostratigraphic scale whereas with isotope geology the geochronometric scale has been more important. Whereas all methods may be useful in connection with either scale, each of which has its distinctive advantages, metazoan fossils and precise biostratigraphy are limited to the last seventh of Earth history and that has resulted in the almost exclusive use of the chronostratigraphic scale by Phanerozoic geologists whereas Precambrian geologists use mainly the geochronometric scale.

I suggest that this is neither a necessary nor a useful restriction and that more active steps should be taken to extend the use and range of each scale independently. Until that is done we are liable to use a hybrid scale^{7,8}—Precambrian geochronometric and Phanerozoic chronostratigraphic—the latest Precambrian division being a hybrid with its beginning defined in years (whatever that means in terms of geological history) and its end defined in an initial Cambrian stratotype (whatever its age in years). This hybrid needs rooting out along with the many other "weeds in the Precambrian rock garden"⁹. It inhibits the proper development and use of each scale if each, instead of coexisting with the other, serves only one part of a single scale⁸. In parallel they supplement each other and invite mutual calibration¹⁰.

Extension of the chronostratigraphic scale

For some time the methods so successful in Phanerozoic stratigraphy have been applied to Precambrian rocks, and

Table 1 Chronostratigraphic scale

Eon	Era	Period	Lower divisions	Approximate position on geochronometric scale (Ga)
Phanerozoic	Cenozoic	-----		-0.065 ±
	Mesozoic	-----		-0.225 ±
	Palaeozoic	-----		
		Cambrian	-----	? -0.57 ±
		†	-----	
		Ediacaran*	-----	? -0.65 ±
		†	-----	
		Varangian*	-----	? -0.69 ±
		†	-----	
		Sturtian*	-----	? -0.75 ±
Adelaidean*	Karatani*	-----	†	-0.75 ±
	(U Riphean)	-----		
		?	-----	? -0.95 ±
	Yurmatin*	-----		? -1.35 ±
	(M Riphean)	-----		
and others	Burzyan*	-----		? -1.5 ± 0.15
	(L Riphean)	-----		
and others	and others	and others		

* Familiar names adapted to this scheme for illustration

† Reference point in boundary stratotype

Table 2 Geochronometric scale with Earth time divisions

Ga	'aetas'	'aevum'	Approximate position on chronostratigraphic scale
0	Latest		Cenozoic and Mesozoic
-0.25	Late	(Novum tempus)	
-0.5	Middle	Novotemp (? Novot)	Palaeozoic
-1.0	Early	(*Late PC)	Precambrian
-1.5	Late	(Medium tempus)	
-2.0	Middle	Mediotemp (? Mediot)	
-2.5	Early	(*Middle PC)	
-3.0	Late	(Antiquum tempus)	
-3.5	Middle	Antiquotemp (? Antiquot)	
-4.0	Early	(*Early PC)	
-4.5	Late	(Priscum tempus)	
	? Middle	Priscotemp (? Priscot)	
	? Early		

* Informal names and divisions of Precambrian time as proposed by IUGS Precambrian Subcommittee and borrowed here to show that the divisions should correspond to named divisions of the geochronometric scale. But the boundaries of these divisions have yet to be decided, and may not correspond to the values suggested here, in which case this scheme might be modified accordingly.

Precambrian divisions along these lines have been proposed¹¹⁻¹⁴. Indeed, the time is ripe, in connection with the standardisation of the initial Cambrian boundary¹⁵, similarly to define some earlier divisions¹⁶. These should not be defined in terms of general events (such as the occurrence of plant life or glaciation), even though that may provide hope of good correlation. Table 1 indicates how it could be achieved. It would be necessary to decide on a classification, a nomenclature and a standardisation (at reference points in selected boundary stratotypes). Table 1 illustrates these points.

New classification and nomenclature of the geochronometric scale

At the same time there is pressure from workers concerned with Precambrian geology to improve the geochronometric scale by defining named divisions longer than a million years. Here, again, it is necessary to decide on a classification, a nomenclature and a standardisation.

The example shown in Table 2, like that shown in Table 1 is designed rather to illustrate the general principle than to press its particular merits, so that out of criticism an agreed scale may emerge.

An hierarchy with divisions larger than a year would be useful as many events have ages which would be easier to express in divisions of a longer duration. I suggest two divisions. The larger are compounded of smaller divisions of 0.5 Ga ($= 0.5 \times 10^9$ yr). I considered a scheme with divisions at -1.0, -2.0, -3.0, -4.0 Ga (that is, a gignennial system) but rejected it because it might have put into words a reversed system as confusing as centuries BC. I then tried a scheme with four names for spans of up to 1.5 Ga and a threefold subdivision of each—early, middle and late (or with subscripts 1, 2 and 3, as in Russian stratigraphic abbreviations).

I chose the division at -2.5 Ga because it is probably the

most popular point for dividing Precambrian time⁹. The 1.5 Ga division may be termed an *aevum* (eon) and the 0.5 Ga span an *aetas* (age).

Existing nomenclature is Precambrian-orientated, loaded with associations, and ambiguous or hybrid, so new terms are needed for the new task. They will need to be clear and convenient if they are to be used in preference to the obvious numerical alternative.

Most stratigraphic roots are derived from the Greek (chron, period, era, eon, eo, proto, archaeo, palaeo, meso, neo and kaino) possibly because they combine more elegantly than Latin roots. Latin has hardly been used so far, so I suggest that Greek be used with the chronostratigraphic scale and Latin be used with the geochronometric scale (with the exception of 'geochronometry'). I considered several Latin roots: *ortus* and *natus* (born), *origo* (origin), *priscus* (ancient), *matutinus* (early), *vetustus* and *antiquus* (old), *medius* (middle), *serus* (late) and *novus* (new). Words like *novus* may be combined in the substantival form *novum tempus* (novotime) and the adjectival form *novotemporalis* (novotemporal).

I suggest that for general international use the abbreviated form novotemp (or novot) be used. Many scientists casually use adjectives as nouns: thus, 'The Precambrian' is a common abbreviation for the Precambrian rocks, history, time, research, or whatever noun is appropriate in context. So in this way the single adjectival abbreviated form might serve as an international technical name without inflection.

The particular names suggested here are not necessary to the scheme and others may be devised. Greek roots are easier and if used with a distinctive suffix may be more acceptable if defined and agreed as a whole.

Standardisation will be simply in years and there is a consensus that very round figures be used so as to avoid any implication of definition by historical events.

Each subdivision (*aetas*) is defined as a precise periodic multiple of 500 Ma. Strictly, years BP go back from a datum at 1950 but it is suggested that, unless a subdivision Latest Novotime beginning at 1950 be used, Late Novotime be extended. Similarly, at the beginning of Priscotime a different procedure may be considered.

The numerical expression of the scale could be distinguished by the negative sign from the same value used as a duration, in preference to the use of BP (see ref. 17).

The magnitude of time span of the proposed divisions is suitable for general discussion of the physical and chemical evolution of the Earth and indeed of the Solar System, so providing a convenient planetary descriptive scale. Cosmologists may advise on how to define the early spans of Priscotime if it be desired to consign the early history of the Earth mostly to its third subdivision.

Scales independent of history

These suggestions distinguish clearly between the chronostratigraphic and geochronometric scales and also avoid the restriction of the latter to Precambrian time ('Precambrian' belongs to the former). Nevertheless, the geochronometric scale will serve Precambrian time with eight of its nine or ten subdivisions. If a hybrid division be required it can be expressed in hybrid form, namely, Precambrian Novotemp (or PC Novot).

It is desirable to avoid names for the geochronometric scale that are associated with the confused history of Precambrian stratigraphy. There are, however, a few rather strong weeds in Rankama's 'rock garden' that will be difficult to eradicate, namely Archaeozoic, Proterozoic, and Eo-Cambrian. These may usefully continue without definition. There is an advantage in retaining a generally understood nomenclature which is intentionally vague so that when an uncertain age is expressed the old names can be used. The new scales would thereby be spared misuse.

In the meantime, if Early, Middle and Late Precambrian time be used as informal names (as suggested by Rankama through the Precambrian Subcommittee) the values chosen

for calibration should also be used for dividing Antiquot, Mediot, and Novot

Finally, agreement on the two artificial scales suggested merely improves the equipment for dealing with the real problems of geology requiring the use of all kinds of phenomena for correlation, and describing their evolution on either scale. Phenomena also need some regulation as to their terminology and nomenclature but their history can never be regulated, and this is the fundamental distinction between the framework and phenomenon categories⁶. Both scales are needed to investigate geological history and to do so they should avoid dependence on it.

Professor C O Brink contributed the classical expertise in proposing the new Latin terminology

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Ethidium bromide inhibits appearance of closed circular viral DNA and integration of virus-specific DNA in duck cells infected by avian sarcoma virus

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The DNA of avian sarcoma virus assumes a closed circular configuration before integration into the host cell chromosomal DNA. Ethidium bromide reduces the formation of superhelical viral DNA and concurrently blocks integration of the viral genome. Inhibition of integration of viral DNA results in the inhibition of virus replication.

FOLLOWING infection of permissive cells by RNA tumour viruses, a DNA intermediate is transcribed from viral RNA (ref. 1) and subsequently integrated into the host cell genome². Although synthesis of viral DNA seems to be crucial in the virus life cycle, it has not been established whether virus production depends on integration of viral DNA. Since no viral mutants defective in integration have been isolated, alternative approaches have been sought to document the necessity of integration for virus replication. One such approach is to block integration with chemical inhibitors. We show here that ethidium bromide (EB), a phenanthridine dye, allows synthesis of normal quantities of viral DNA but blocks its integration into the host DNA, probably by interfering with the formation of covalently closed circular DNA. This closed circular molecule, which can be demonstrated in alkaline sucrose and equilibrium density gradients, is presumably the form of viral DNA just before its integration. We also present evidence for a direct relationship between the amount of viral DNA integrated and the amount of virus-specific RNA produced by the infected cell.

EB affects viral DNA integration

Peking duck cells were pretreated for 6 h with EB (1.0 $\mu\text{g ml}^{-1}$) and then infected with B77 avian sarcoma virus in the presence of the inhibitor. Forty hours after infection, DNA was isolated from untreated (control) and EB-treated cells and assayed for virus-specific nucleotide sequences by testing its capacity to influence the reassociation of double-stranded viral DNA synthesised *in vitro*³. In the experiment presented in Fig 1a

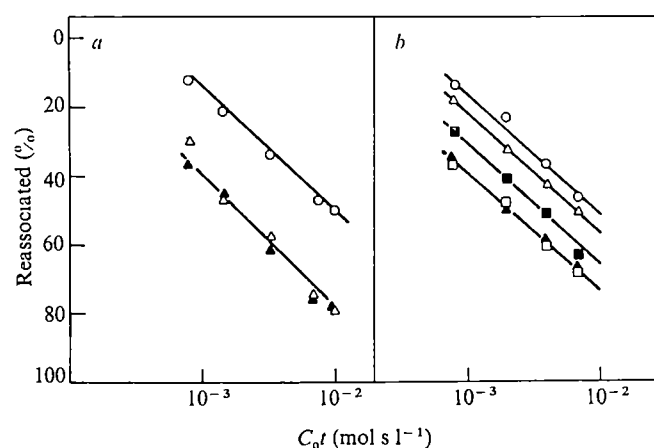


Fig 1 Effect of EB on integration of viral DNA. Peking duck cells (prepared from 12–14-d-old embryonated eggs) were grown to a density of $4\text{--}5 \times 10^6$ per 100 mm plate. Half of the cells were infected with B77 virus at a multiplicity of infection of 3 in the presence of polybrene ($2 \mu\text{g ml}^{-1}$) and the other half was pretreated for 8 h with EB ($1.0 \mu\text{g ml}^{-1}$) and then infected in the presence of the inhibitor. After 40 h incubation, high molecular weight DNA was extracted and tested for integration as described by Varmus *et al*³. To detect the presence of virus-specific nucleotide sequences, radioactive duplex virus-specific DNA (representing about 30% of the total viral genome²) was denatured and reassociated in the presence of various samples of cellular DNA. Acceleration of the reassociation of the radioactive DNA indicates the presence of virus-specific nucleotide sequences in the cellular DNAs. Each reaction mixture contained radioactive DNA (1.6 ng ml^{-1} , $15,000 \text{ cpm ng}^{-1}$). Reassociation was monitored by fractionation on hydroxyapatite. *a*, (○) normal duck DNA (1.6 mg ml^{-1}), DNA (Δ) from B77-infected control (1.6 mg ml^{-1}) or (▲) EB-treated (1.5 mg ml^{-1}) duck cells. *b*, (○) normal duck DNA (1.6 mg ml^{-1}), (□) network DNA (1.3 mg ml^{-1}) or (■) supernatant DNA (1.1 mg ml^{-1}) from control cells or (Δ) network DNA (1.3 mg ml^{-1}) or (▲) supernatant DNA (0.5 mg ml^{-1}) from EB-treated cells.

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Table 1 Inhibition of integration by EB

Sample	Total DNA		Network DNA		Supernatant DNA	
	$C_{0t_{1/2}}$	copies per cell	$C_{0t_{1/2}}$	copies per cell	$C_{0t_{1/2}}$	copies per cell
Uninfected	1.0×10^{-2}	0.00	9.2×10^{-3}	0.00	9.2×10^{-3}	0.00
Infected untreated	1.7×10^{-3}	1.20	2.0×10^{-3}	1.10	3.7×10^{-3}	0.60
EB-treated	1.7×10^{-3}	1.20	7.0×10^{-3}	0.15	2.0×10^{-3}	2.80

$C_{0t_{1/2}}$ values were obtained from reassociation kinetics given in Fig. 1. Copy numbers were computed from $C_{0t_{1/2}}$ as described by Gell et al.¹⁷

and Table 1, 1.2 copies per cell of viral DNA were synthesised in either the presence or absence of EB, indicating that EB has no effect on the net synthesis of viral DNA. In some experiments (data not shown), 15–20% inhibition was observed with this dose of EB. Since virtually normal levels of viral DNA are synthesised, these data indicate that, in contrast to the results of studies *in vitro*⁴, EB does not inhibit the action of reverse transcriptase in the infected cell.

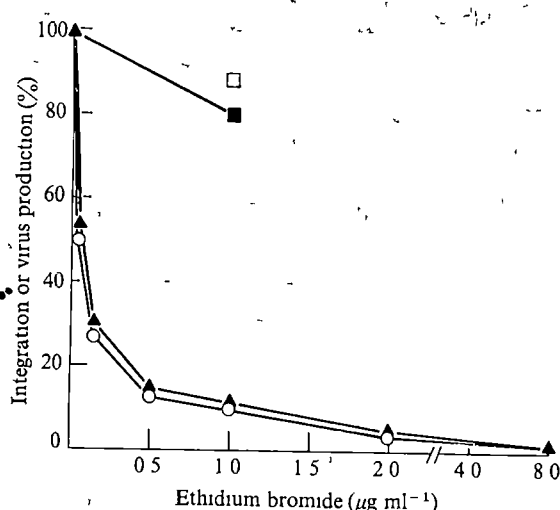


Fig. 2 Duck cells, either untreated or pretreated for 8 h with EB, were infected with B77 virus at a multiplicity of infection of 5 in the absence or presence of the inhibitor. After 38 h, high molecular weight DNA was isolated and assayed for integration as in Fig. 1. Also, one plate from each batch was labelled with ^3H -uridine ($16 \mu\text{Ci ml}^{-1}$, 27 Ci mmol^{-1}), and fluids were collected and banded in equilibrium sucrose gradients as described in the legend to Table 1. One plate from duck cells infected 7 d previously was labelled similarly after 24 h (\square) or 48 h (\blacksquare) treatment with EB. \circ , Integration of viral DNA, \blacktriangle , virus production.

Integration of viral DNA into the cell genome was, however, affected by EB. The DNA was tested for integration by a recently described technique². Under appropriate conditions, reassociated cell DNA reassociates to form network-like structures, viral DNA covalently linked to strands of cell DNA appears in these structures. Network DNA from cells infected in the absence of EB accelerated the rate of reassociation of

labelled viral DNA by a factor of 5, whereas the network DNA from EB-treated cells had only a marginal influence on the reassociation (Fig. 1b). The data indicate that less than 15% of viral DNA (0.15 copy compared with 1.1 copies in the control) was integrated in the presence of EB (Table 1). As expected, DNA from EB-treated cells that had not entered into networks markedly decreased the $C_{0t_{1/2}}$ of labelled viral DNA, indicating considerable (six- to eightfold) enrichment of virus-specific sequences in the supernatant which included all unintegrated viral DNA. From these results we conclude that EB does not inhibit viral DNA synthesis, but prevents integration of viral DNA into host cell DNA.

Similar results were obtained with EB ($0.5 \mu\text{g ml}^{-1}$) but with still lower concentrations, the extent of inhibition decreased significantly (Fig. 2). Concentrations of EB above $1.0 \mu\text{g ml}^{-1}$ not only blocked integration but also suppressed synthesis of viral DNA. For example, in the presence of EB at $2.0 \mu\text{g ml}^{-1}$ only 45% of control levels of viral DNA was synthesised, and integration was totally inhibited. The suppression of viral DNA synthesis by the higher doses of EB could be caused by cytotoxic effects of the drug. Doses of $1.0 \mu\text{g ml}^{-1}$ or less had little or no effect on cell growth during a 48-h exposure (see below, Table 2), whereas higher doses severely depress cell growth (R. V. G., B. W. J. M., J. M. B., and H. E. V., unpublished).

EB and supercoiled viral DNA

Recently, we have shown that at least 10–20% of viral DNA exists in a covalently closed duplex circular form 8–10 h after infection of duck cells by avian sarcoma virus^{5,6}. Since EB binds to duplex DNA and causes unwinding of the molecule, we proposed that EB might intercalate with double-stranded viral DNA to prevent or reverse the formation of supercoiled DNA (form I) and thereby inhibit integration. The following evidence supports this notion.

Integration of viral DNA into the cell genome starts about 10 h after infection². If formation of supercoiled viral DNA is required for integration, we expect maximum levels of this species before integration. Therefore, untreated and EB-treated duck embryo fibroblasts were infected for 9 h in the absence and presence of the dye and cells were fractionated according to the procedure described by Hirt, which has been used to separate papovavirus DNA from host cell DNA⁷. The DNA in the Hirt supernatant was analysed for the presence of closed circles in an alkaline sucrose gradient. Three distinct peaks of viral DNA, detected by hybridisation to cDNA (DNA complementary to viral genome), were observed in both control and EB-treated supernatant fractions. About 18% of the

Table 2 Effect of EB on virus production in B77-infected duck cells

	Cells per 100-mm plate	Viral DNA copies per cell	Integration (%)	^3H -uridine incorporation (c.p.m.)	FFU ml ⁻¹
Control	8.7×10^6	1.7	100	3,095	7.5×10^4
EB	7.2×10^6	1.5	17	417	8.5×10^3

Duck cells (4×10^6 per 100 mm plate), untreated or pretreated for 4 h with EB ($1.0 \mu\text{g ml}^{-1}$) were infected with B77 virus at a multiplicity of infection of 5 for 40 h in the absence or presence of EB. High molecular weight DNA was isolated and assayed for integration as described in the legend to Fig. 1. One plate from each group was labelled with ^3H -uridine ($8 \mu\text{Ci ml}^{-1}$, 27 Ci mmol^{-1}) for 8 h and the growth fluids were banded in an equilibrium sucrose gradient (15–55% sucrose in 0.1 M NaCl, 10 mM Tris-HCl, pH 7.4, 10 mM EDTA, SW41 rotor, 3.5 h at $40,000 \text{ rpm}$ and 4°C). The tritium label banding at a density of $1.15\text{--}1.17 \text{ g ml}^{-1}$ was taken as the amount incorporated into the virion RNA. Aliquots of growth fluids from control and EB-treated samples were also assayed for virus production by focus formation on chicken cells. FFU, Focus-forming units.

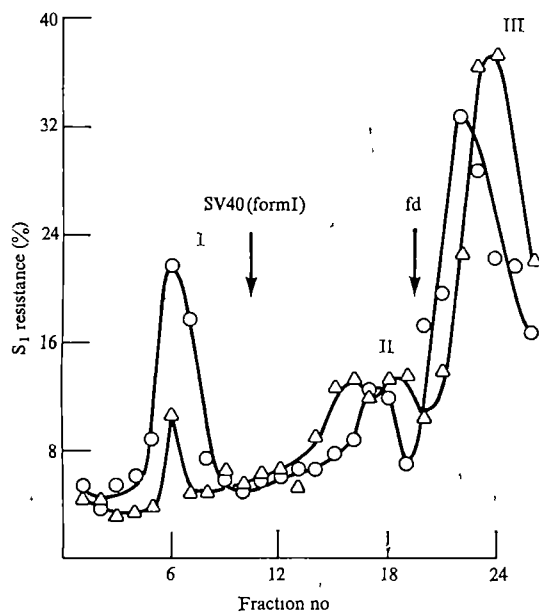


Fig 3 Sedimentation of viral DNA in alkaline sucrose gradient. Duck cells, either untreated or pretreated with EB ($0.25 \mu\text{g ml}^{-1}$) for 14 h, were infected with B77 virus at a multiplicity of infection of 4–5 in the absence or presence of EB ($1.0 \mu\text{g ml}^{-1}$). After 9 h, cells were collected and fractionated as described by Hirt⁷. DNA was isolated from the Hirt supernatant by treatment with pronase followed by phenol extraction. After ethanol precipitation, the DNA was treated with pancreatic RNase in 10 mM Tris-HCl (pH 7.4)–10 mM EDTA, re-extracted with phenol, ethanol precipitated and analysed on a 5–20% sucrose gradient in 0.3 M NaOH, 0.7 M NaCl and 1 mM EDTA. Centrifugation was for 3 h in a SW41 rotor at 40,000 r.p.m. and 20°C . Fractions were collected from the bottom of the gradient, supplemented with calf thymus DNA ($50 \mu\text{g ml}^{-1}$) to serve as carrier, and then heated for 2 h at 80°C to reduce the size of DNA. The fractions were neutralised and precipitated with ethyl alcohol. DNA was collected by centrifugation and assayed for virus-specific sequences using ^3H -labelled cDNA ($1,270 \text{ c.p.m. per fraction}$, $20,000 \text{ c.p.m. ng}^{-1}$). Annealing was for 4 d at 68°C in 0.6 M NaCl in 40 μl volume. Resistance of ^3H -cDNA to S1 nuclease was determined as described previously¹⁸. Under these conditions of annealing, the reaction was linear from 0.05 to 0.5 ng ($5\text{--}30\%$ S1 resistance) of viral DNA¹⁶. This permits quantitation of virus specific DNA in an individual sample. Supercoiled SV40 (form I, 53S) and single-stranded circular fd DNA (20S) were run in parallel gradients. Sedimentation is from right to left. \circ , Control, \triangle , EB

virus-specific DNA sedimented at 62–65S (peak I, Fig 3), this species represents supercoiled viral DNA^{5,6} (R V G *et al*, unpublished). More than 60% of the viral DNA is much smaller in size (molecular weight $< 1.0 \times 10^6$, peak III). About 10–15% sedimented at approximately 22S, corresponding to a molecular weight of 3×10^6 to 3.5×10^6 (equivalent to the size of the 35S subunits of the viral genome¹). EB suppressed the formation of closed circles (peak I) by a factor of 4–5, without affecting the other forms (peaks II and III, Fig 3), less than 0.1 ng out of 2.8 ng was found as supercoiled DNA in the EB-treated sample compared with 0.5 ng out of 2.4 ng in the control (see legend to Fig 3). These results suggest that EB specifically blocks or reverses the formation of supercoils. Further evidence in support of this comes from the following experiment.

Supercoiled DNA (form I) binds less EB than open circular (form II) or linear (form III) duplex DNA⁸. Consequently, form I DNA bands at a higher buoyant density (about 0.035 g ml^{-1} greater than forms II and III) in CsCl-EB equilibrium density gradients⁸. Analysis of the Hirt supernatant fraction from duck cells infected for 9 h with B77 virus revealed that two separate peaks of viral DNA could be resolved with a density difference of about 0.036 g ml^{-1} , under similar conditions

SV40 forms I and II were, as expected, separated by a density difference of 0.038 g ml^{-1} . The hybridised viral DNA banding at higher density is consistent with a closed circular molecule.

In agreement with the results shown in Fig 3, EB suppressed the formation of only the supercoiled DNA by more than 80% (Fig 4). Computation of the amount of viral DNA from percentage hybridisation¹⁶ indicates that comparable amounts of viral DNA were synthesised in EB-treated and untreated cells (about 6 ng), but less than 0.2 ng of the total banding as form I in the EB-treated sample as opposed to about 1.3 ng in the control.

To demonstrate unequivocally that the DNA binding at the higher density in Fig 4 is closed circular DNA, we showed that its density in CsCl-EB gradients could be altered by limited exposure to pancreatic DNase. Viral DNA from the Hirt supernatant of cells infected for 9 h was centrifuged to equilibrium in a CsCl-EB gradient, 15–20% of the total viral DNA cosedimented with PML21 form I (Fig 5a). When single-strand breaks were introduced by pancreatic DNase, both the

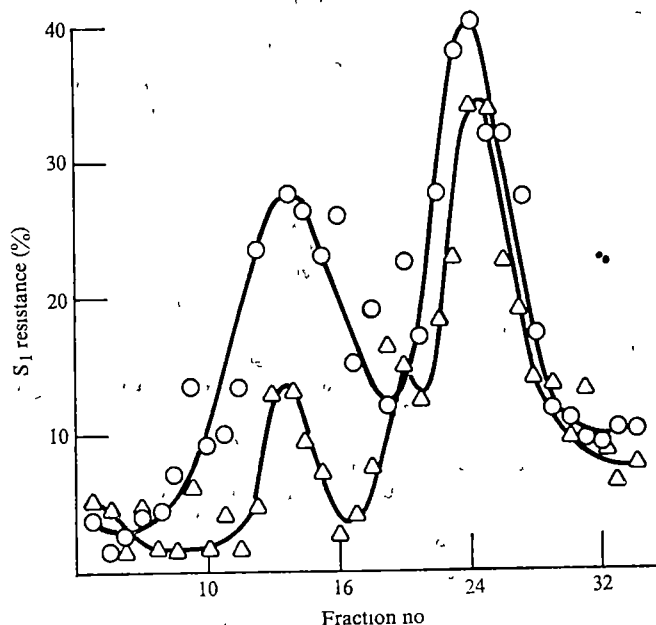


Fig 4 Equilibrium gradient centrifugation of viral DNA. The remaining portions of the Hirt supernatants from duck cells infected with B77 virus in the presence or absence of EB (see legend to Fig 3) were analysed in a CsCl-EB gradient. To a 6-ml sample in 10 mM Tris-HCl–3 mM EDTA 6 g, CsCl and EB ($300 \mu\text{g ml}^{-1}$) were added and centrifuged for 66 h in a type 40 rotor at 33,000 r.p.m. and 20°C . ^3H -labelled SV40 DNA was also centrifuged in a parallel gradient. Fractions were collected and assayed for virus-specific DNA with ^3H -cDNA ($1,330 \text{ c.p.m.}$, $20,000 \text{ c.p.m. ng}^{-1}$) as described in Fig 3. The viral DNAs corresponding to supercoiled and open or linear forms banded at 1.594 and 1.558 g ml^{-1} respectively. SV40 form I has a buoyant density of 1.590 g ml^{-1} under similar conditions and banded between fractions 8 and 14. \circ , Control, \triangle , EB

viral DNA and PML21 marker DNA shifted to a lighter density peak (Fig 5c), corresponding to form II or III. Re-banding of the same material without DNase treatment showed that form I DNA was still intact (Fig 5b). This effect of pancreatic DNase on the density shift of viral DNA strongly supports our conclusion that at least a portion of the viral DNA synthesised *in vivo* exists as a closed circular molecule.

We have consistently observed that a measurable amount of viral DNA bands at a position intermediate to the heavy and light density peaks of DNA, thereby broadening the bandwidth of form I DNA (Figs 4 and 5). The density pattern of this DNA is unchanged by treatment with pancreatic DNase. The nature of this species is at present unclear and is being investigated.

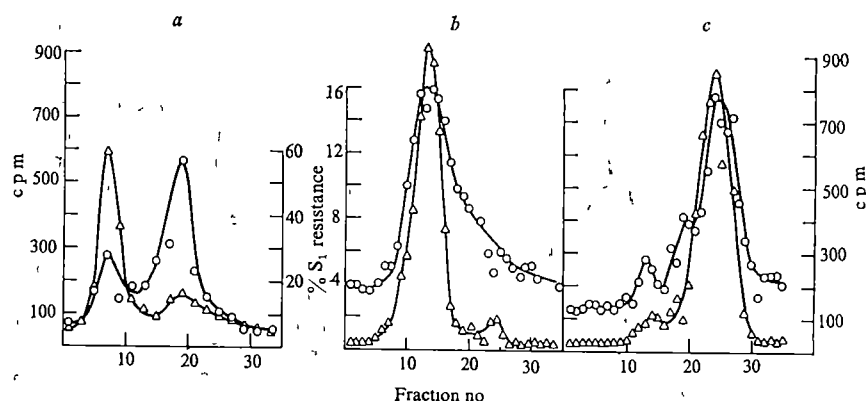


Fig. 5 Conversion of form I DNA to form II by pancreatic DNase. Duck cells were infected with B77 virus at a multiplicity of infection of 6 and after 9 h, cells were collected and fractionated as described by Hirt⁷. DNA was isolated from the Hirt supernatant as described in the legend to Fig. 3. ³H-labelled PML21 plasmid DNA (molecular weight 7.1×10^6 dalton) was added to the supernatant DNA and banded in an equilibrium CsCl-EB gradient (6.3 g CsCl in 6.8 ml sample containing EB at $300 \mu\text{g ml}^{-1}$). a, One-fourth volume from alternate fractions was hybridised to ³²P-labelled cDNA as in Fig. 3. The DNA banding at heavy density (fractions 4-12) was pooled and EB was removed by passing through Dowex-AG50. Calf thymus

DNA ($60 \mu\text{g}$) was added and the pooled sample was dialysed against 20 mM Tris-HCl to remove CsCl. The sample was divided into two equal portions, one portion served as control (b) and the other half was treated with pancreatic DNase (c) (Worthington) in 10 mM MgCl₂, 20 mM Tris-HCl, pH 7.4, 10 mM NaCl, bovine serum albumin ($30 \mu\text{g ml}^{-1}$) and $5 \times 10^{-5} \mu\text{g}$ DNase for 20 min at room temperature. The reaction was stopped by the addition of 50 mM EDTA and the samples were centrifuged to equilibrium in CsCl containing EB ($300 \mu\text{g ml}^{-1}$). The conditions of centrifugation and hybridisation were as described in Fig. 4. O, Percentage hybridisation to ³²P-cDNA, Δ , ³H-labelled PML21.

EB inhibits virus production

To explore the relationship between integration of viral DNA and virus production, the following experiments were performed. Cells were pretreated with $1.0 \mu\text{g ml}^{-1}$ EB for 6 h and infected for 40 h in its presence. DNA was then isolated and tested for the presence and integration of virus-specific nucleotide sequences. Virus production was monitored by the incorporation of ³H-uridine into the RNA of mature virus released into the medium and by assay of the culture medium for focus-forming activity. In addition, the rate of synthesis and concentration of virus-specific RNA in the infected cells were measured by hybridisation of cellular RNA to virus-specific complementary DNA (cDNA) synthesised *in vitro*⁹. The results from a typical experiment (Table 2) show that EB ($1.0 \mu\text{g ml}^{-1}$) suppressed integration and virus production coordinately by a factor of 6-8. Measurement of virus-specific RNA in the EB-treated cells by hybridisation to ³H-labelled cDNA gave similar results (Table 3). EB at $1 \mu\text{g ml}^{-1}$ reduced the concentration of viral RNA by about 80% as early as 24 h after infection, and the degree of inhibition did not change appreciably during 72 h. Substantial amounts of input viral RNA, however, result in high levels of background and thus might obscure the total effect of EB on viral DNA synthesis in this type of experiment. This problem can be overcome by studying the effect of EB on the *de novo* synthesis of virus-specific RNA. Therefore, 48 h after infection, cells were pulse-labelled with ³H-uridine, and RNA was isolated and analysed for virus-specific sequences by hybridisation to

unlabelled cDNA as described previously¹⁰. In these conditions EB inhibited synthesis of virus-specific RNA by approximately 75% (Table 4), the reduction being proportional to the reduction in the amount of viral DNA integrated into cell DNA.

The data in Fig. 2 further substantiate the conclusion that virus production depends on integration of viral DNA. There is a good correlation between the extent of viral DNA integrated and the magnitude of virus production. The inhibition of viral replication is not a simple consequence of the effect of EB on cell growth for there was only a barely perceptible decrease in the number of cells (Table 2). Moreover, the effect of EB on virus production is appreciable only if the inhibitor is added before integration. Addition of the dye ($1.0 \mu\text{g ml}^{-1}$) to cells after 24 h of infection (data not shown) or to fully infected cells producing virus (Fig. 2) did not inhibit virus production. These results strongly suggest that integration of viral DNA into the cell genome is mandatory for virus production.

Our results are different from those obtained by Reichert and Hare¹¹, who demonstrated that virus replication is unaffected by EB at $1.0 \mu\text{g ml}^{-1}$. Substantial differences in the experimental protocols used might partly explain this dissimilarity. Bader¹², on the other hand, found that this concentration of EB reduced viral RNA synthesis by 60% either in JSL-V5 cells infected by the Rauscher strain of murine leukaemia virus or in chick embryo fibroblasts infected by Rous sarcoma virus. We observed similar inhibition of virus production in chick cells (70%) as opposed to duck cells (80-85%) in the presence of EB ($1.0 \mu\text{g ml}^{-1}$).

Table 3 Synthesis of B77 viral RNA as analysed by hybridisation to cDNA

Time after infection (h)	Integration (%)		$C_{t_{1/2}}$ (mol s ⁻¹)		Virus-specific RNA (%)		Inhibition (%)
	Control	EB	Control	EB	Control	EB	
24	—	—	5.5×10^2	2.5×10^3	0.004	0.0008	78
48	100	15	2.4×10^2	1.3×10^3	0.009	0.0015	84
72	—	—	1.1×10^2	6.0×10^2	0.020	0.0030	82

Duck cells were pretreated with EB ($1.0 \mu\text{g ml}^{-1}$) for 2 h and infected with B77 virus in the presence of the inhibitor. Control cells did not receive EB. At the indicated times, cells were collected and RNA was extracted with SDS-pronase-phenol¹⁸. The RNA was then hybridised to ³H-labelled cDNA synthesised *in vitro*. Details of this technique were published elsewhere¹⁸. DNA was tested for integration as described in the legend to Fig. 1. The amount of virus-specific sequences in the total cell RNA was calculated from the $C_{t_{1/2}}$ (2×10^{-2}) obtained with purified 70S virion RNA and cDNA¹⁸. Avian sarcoma virus-transformed cell RNA has a $C_{t_{1/2}}$ ranging from 2 to 10 suggesting that 0.2-1% of the total RNA is virus-specific.

Table 4 Effect of EB on virus-specific RNA

Sample	Hybridisation input		Radioactivity in hybrid (%) [†]		Virus-specific RNA (%) [§]
	³ H*	³² P†	³ H	³² P	
Control	470,655	1042	0.28	61.0	0.46
EB-treated	373,291	1169	0.08	65.0	0.12

*Duck embryo cell monolayers were pulse-labelled for 100 min with ³H-uridine (1 mCi ml⁻¹ in nucleoside-free medium) 48 h after infection with B77 virus in the presence or absence of EB (1.0 µg ml⁻¹). At the conclusion of the pulse, total cell RNA was extracted as described previously¹⁸. The specific activity of the purified RNA was 1.0 × 10⁶ c.p.m. µg⁻¹.

†³²P-labelled 70S RNA (specific activity 1 × 10⁶ c.p.m. µg⁻¹) was purified from B77 virus⁹.

‡The hybridisation mixture in a total volume of 10 µl 2 × SSC (NaCl (0.15M)—Na citrate (0.015M)) contained the indicated amounts of ³H-cell RNA and ³²P-70S B77 RNA plus 24 µg yeast RNA and either 30 ng unlabelled cDNA synthesised using Prague-C Rous sarcoma virus or 30 ng phage λ DNA as control. After annealing for 24 h at 68°C, the percentage of RNA present in RNA-DNA hybrids was determined essentially as described by Parsons *et al.*¹⁰ Results were corrected for a background of 0.3% in control samples.

§Calculated as ³H hybridised (%) / ³²P hybridised (%) × 100.

The claims that virus production occurs in mitochondria^{13,14} have been challenged¹². Since EB selectivity inhibits mitochondrial RNA transcription and DNA replication¹⁵, our results could be interpreted to mean that virus production occurs in mitochondria, or that a function involved in mitochondrial DNA replication also participates in viral DNA replication and integration. This seems unlikely, however, for two reasons: (1) although viral DNA synthesis occurs in the cytoplasm shortly after infection, virus-specific DNA subsequently migrates into the nucleus and integrates with chromosomal DNA¹⁸, and (2) mitochondrial DNA, isolated from B77-transformed duck cells, did not hybridise to virus-specific ³H-labelled cDNA (R.V.G. unpublished). These results argue against the possibility that mitochondria are involved in virus replication and support Bader's results¹².

Molecular events before viral DNA integration

We have presented evidence favouring the following scheme for the synthesis of viral DNA after infection by avian sarcoma viruses. Viral DNA is made in the cytoplasm during the first 6 h after infection as a linear (or open circular) duplex molecule of a length similar to that of a subunit of the viral RNA genome (molecular weight 3.0 × 10⁶)^{5,6,16}, these molecules are unusual in that the predominant form contains full length strands complementary to the viral genome ('minus' strand) but relatively short 'plus' strands of DNA²⁰ (less than 1.0 × 10⁶ daltons, H.E.V. *et al.*, in preparation). Viral DNA then migrates into the nucleus at about the time that we have observed the supercoiled form of viral DNA¹⁶. Under ordinary circumstances, all or a substantial portion of viral DNA becomes integrated into the cell genome between 9 and 24 h after infection². The

presence of EB suppresses the accumulation of closed circular viral DNA, presumably by intercalation into DNA duplexes. Since the accumulation of closed circles and the integration of viral DNA are reduced concomitantly and to similar extents, we propose that a circular molecule is the form which undergoes the crossing-over step(s) required for integration. If our hypothesis is correct, the mechanism of integration for RNA tumour viruses is fundamentally similar to that for several DNA tumour viruses¹⁹. The molecular mechanism by which circles are formed remains uncertain.

Since viral RNA and progeny virus are not made when integration of viral DNA is blocked by EB, it seems that integration is a mandatory step for full expression of the viral genome. We cannot as yet exclude the possibility that the drug also exerted subtle effects on cell metabolism unrelated to viral events.

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Note added in proof Closed circular viral DNA has also been identified in mouse cells infected by murine leukaemia virus (Gianni, A.M., Smotkin, D., and Weinberg, R.A., *Proc natn Acad Sci*, (in the press)).

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letters to nature

On the planetary theory of sunspots

It has been proposed^{1,2} that sunspot activity is affected by positions of the planets, and calculations have been presented³, which purport to show that planetary tides on the Sun vary in the same way as the sunspot variations. We believe that the apparent agreement of the sunspot cycle with planetary tidal effects is an artefact of the calculation.

The calculation in question³ was used to compute the absolute value of the difference in tidal potential between Earth-Venus

conjunctions and oppositions at the sub-Jupiter solar point. The effect of Mercury, one of the strongest tide-raising planets was ignored on the basis that its period is short compared with that of sunspot activity. The absolute value of the tide and the effect of partial line-ups of Venus (or the Earth) with Jupiter were not computed. Furthermore, it is not clear that the absolute difference between opposition and conjunction tidal potentials has any physical meaning.

Here we compute the full tidal problem for Mercury, Venus, Earth and Jupiter, the tide-raising planets, taking into account

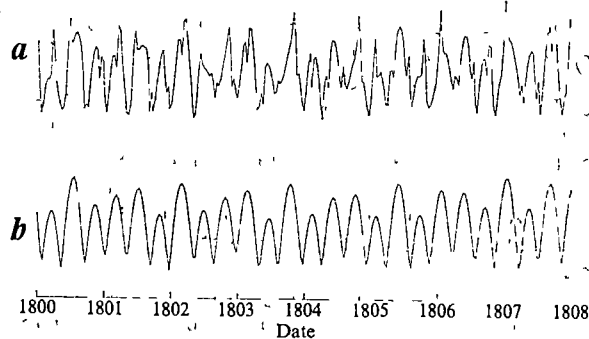


Fig 1 Tidal potential for the years 1800-08 *a*, Full four-planet tidal potential, *b*, tidal potential excluding Mercury. Both scales are identical

the complete orbital elements, including eccentricity, inclination and their variation with time

At any given time, the tidal potential at a given point *M* on the surface of the Sun caused by the planets is proportional to

$$T = \sum \frac{m_i}{d_i^3} (\cos^2 z_i - \frac{1}{3})$$

where m_i is the mass of the planet, d_i its distance from the centre of the Sun and z_i the angle from the planet to point *M* as seen from the centre of the Sun. We restrict ourselves to Mercury, Venus, Earth and Jupiter. Mars, Saturn and the other outer planets can easily be shown to have trivial contributions compared to the above planets

Table 1 Comparison of tidal and sunspot dates

Tidal peak	Sunspot peak	Dts*
1809	1816	-7
1822	1830	-8
1833	1837	-4
1845	1848	-3
1857	1860	-3
1869	1871	-2
1881	1884	-3
1892	1894	-2
1905	1906	-1
1916	1918	-2
1927	1928	-1
1939	1938	+1
1951	1948	+3
1963	1958	+5
1974	1969	+5

* Dts is the difference between tidal and sunspot peaks in years

Tidal peaks are taken from Fig 2 (and its continuation up to the year 2000). Sunspot peaks are from Wood

In the plane of the ecliptic, the potential depends on the longitude ϕ through a first order polynomial of $\sin 2\phi$ and $\cos 2\phi$. Over a period of 10 d (our sampling interval) the Sun rotates about halfway around its axis and therefore any given point attached to its surface is subject to the whole variation of the tidal potential. So we characterised our problem by the maximum value (over ϕ) of this polynomial. We note that Mercury is the slowest planet around the Sun relative to a point on the Sun's surface and its contribution, contrary to previous arguments³ should therefore not be neglected

The positions of the planets were computed from the best available planetary elements⁴ for 65,536 epochs at intervals of 10 d, or roughly 1,800 yr, starting in the year 1800. A fast Fourier transform (FFT) technique was then used to extract the power spectrum for comparison with the solar activity spectrum

Figure 1 shows the tidal potential as a function of time for the years 1800-1808, both including Mercury (upper trace) and excluding it (lower trace). The high frequency effect is due to the eccentricity of Mercury's orbit and has a period of 0.24 yr

Figure 2, an extension of the lower trace in Fig 1, shows the tidal potential for the 25-yr period 1800-1825, excluding Mercury. The long period, beat-type phenomenon (~ 11.9 yr) arises because of the eccentricity of Jupiter's orbit. By contrast with the sunspot cycle, the tidal pattern repeats almost exactly every 11.9 yr and shows no evidence of a beat of ~ 100 yr. Successive peaks in the tidal envelope are of almost exactly the same amplitude. Wood's samples³ (● in Fig 2) have a spacing too large to provide a valid description of the tidal potential even excluding Mercury, such a sampling leads to aliasing of the lower frequencies. Figure 3 shows the power spectrum obtained from an FFT analysis of the whole 1,800-yr four-planet potential. The fundamental periods are those of the alignments of pairs of planets. Alignments of three, or four planets come as beats of these primary values and consequently do not appear on the spectrum. The periods of Jupiter and Mercury and some of the harmonics show up because of the eccentricity of their orbits. The lower frequency part of the spectrum is very flat, Jupiter being the longest period involved

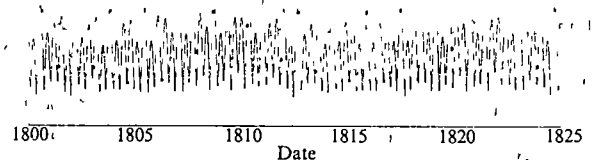
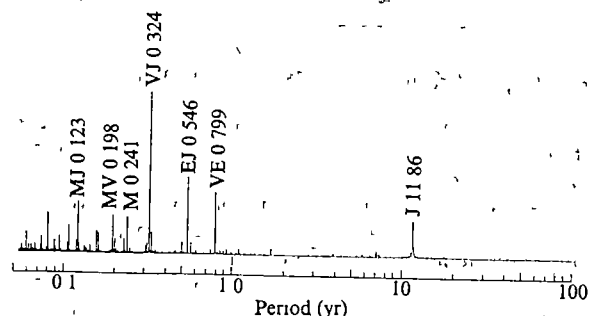


Fig 2 Three-planet tidal potential for the years 1800-1825 (excluding Mercury). The marks (●) show the points used by Wood³

Figure 4 (frequency scaled) shows an enlargement of the lower frequency part of the spectrum, superimposed with Cohen and Lintz's sunspot spectrum⁵. They showed a strong peak occurring at 11 yr, the familiar sunspot cycle, and smaller peaks at about 9.8, 9.58 and 8.3 yr. In addition they demonstrated that the longer period, ~ 180 -yr cycle proposed for the solar sunspot spectrum arises from the beat of the 11 and 9.8-yr cycles and is not an intrinsic periodicity. This removes the basis for one of the other planetary theories of sunspots⁶. Figure 4 shows no planetary peaks at 8.3, 9.8 or 9.58 yr, peaks which are prominent in the sunspot spectrum⁵.

The origin of the 11.08-yr tidal period claimed by Wood³ is not clear. Such a peak does not appear in our spectrum. In Wood's simplified three-planet system, it could only be the consequence of a Jupiter-Earth-Venus alignment. But if 11.08 yr is indeed a multiple of the Venus-Earth 0.799-yr alignment period, it is not a multiple of the Jupiter-Earth synodic period, and therefore is not a fundamental period of the problem. This discrepancy between the tidal and the sunspot activity periods is further demonstrated in Table 1, which gives sunspot peak dates³ and approximate dates of envelope maxima of the tidal potential, excluding Mercury. The average period between envelope peaks

Fig 3 Power spectrum of tidal potential. The horizontal axis is scaled as $\log T$. The labels on the larger peaks identify the periods (yr) of alignments of planets (two-letter label) or the period of the planets themselves (one-letter label)



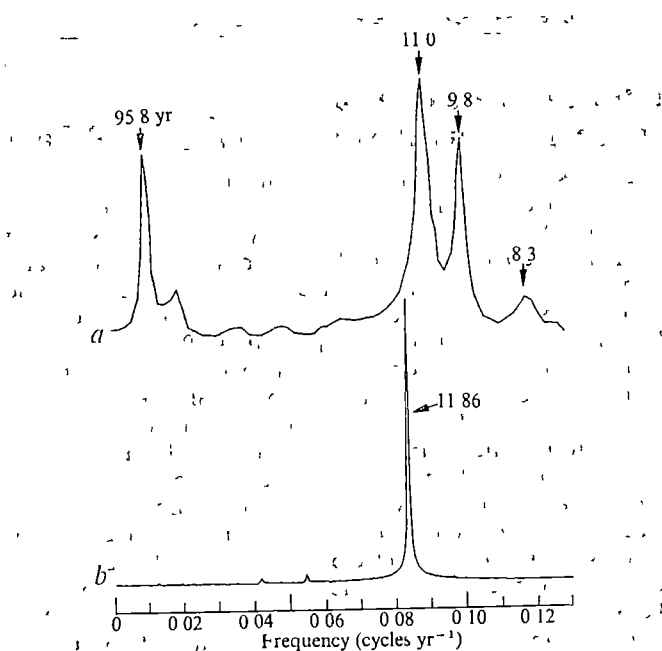


Fig 4 Comparison of the lower frequency part of tidal spectrum (b) and sunspot spectrum (a, from ref 5)

is 11.8 yr, the orbital period of Jupiter. From the beginning of the nineteenth century to the present the discrepancy between tidal peak dates and sunspot peak dates has slipped from approximately -7 yr to $+5$ yr. This is the difference between the 11.86-yr Jupiter period and the average sunspot period, 11.05 yr taken over 165 yr (14 cycles). Over a limited period of time, such as 1892 to 1939, the peak years agree to within a year or two but this is to be expected when comparing two periodic functions of nearly the same period. The next tidal envelope maxima occur in 1987 and 1998. In the incomplete tidal theory³ maxima are predicted for 1982, 1993 and 2003.

A further look at our tidal potential values shows no drastic effect expected in 1982 when planets are supposed to align on the same side of the Sun (see ref. 7). Indeed, better alignment will be achieved in 1990. Even then, no special tidal effect occurs because alignment of the outer planets has no pronounced effects on the tides. Alignment of the tide-raising planets within 10 degrees is a common phenomenon, occurring approximately every 10.4 yr and is not associated with drastic tidal effects.

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The solar spectrum at 8 mm

THE brightness temperature of the quiet Sun has been measured over almost the complete radio spectrum. It ranges from approximately 6,000 K at millimetre wavelengths to over 10^6 K at metre wavelengths. Various compilations of the disk temperatures¹⁻³ had at centimetre and millimetre wavelengths from 10 to 100 GHz ($\lambda=3$ cm to 3 mm) (Fig. 1). A monotonically decreasing brightness temperature

presented by Shimabukuro and Stacey¹ from a model by van de Hulst⁴ is also shown in Fig. 1. Zheleznyakov⁵ has pointed out that the apparent dip in the observed disk temperatures near 50 GHz and at the apparent peak near 70 GHz, if real, would indicate an effective temperature inversion in the solar chromosphere from 3,000 to 3,300 km above the photosphere. Reber³, however, measured the slope of the brightness against frequency curve at 50 GHz and found that the disk temperature is, in fact, decreasing with increasing frequency in nearly perfect agreement with the van de Hulst⁴ model. Reber's method does not depend on an absolute measurement of the brightness temperature but rather on a relative measurement between two closely spaced frequencies, with a single instrument, thereby giving the slope with more accuracy than can be obtained by comparing a number of independent, absolute measurements.

We have measured the slope of the brightness-frequency curve near 36 GHz ($\lambda=8$ mm) to determine whether the slope best matches the van de Hulst model or the observations.

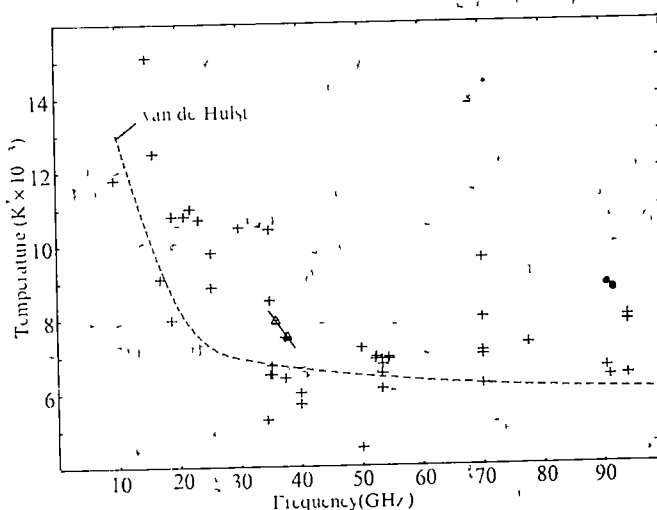


Fig 1 Solar brightness temperatures against frequency. Triangles represent the measured slope of the curve between 36 GHz and 38 GHz.

The observational method used was similar to that of Reber's³ in which the new Moon is used as a calibration source. The ratio of the brightness temperatures of the Sun and Moon is given by

$$\frac{T_s}{T_M} = \left(\frac{T_A(\text{Sun}) - T_A(\text{sky})}{T_A(\text{Moon}) - T_A(\text{sky})} \right) \quad (1)$$

where T_M = brightness temperature of the Moon, T_s = brightness temperature of the Sun, T_A = measured antenna temperature of the Sun, Moon or sky, provided that the solid angle of the antenna beam is smaller than that of the Sun or Moon, and that all measurements are reduced to the same zenith angle. The Sun-Moon ratio is independent of any knowledge of the antenna gain, atmospheric absorption and re-emission or radiometer calibration constants, provided that those parameters remain constant over an observation.

The ratio T_s/T_M was measured on 21 d within 3 d of new Moon at 36.0 GHz and 37.75 GHz, with a Dicke switched, superheterodyne radiometer. The half power beamwidth was $14'$. Two local oscillators were installed in the radiometer so that the frequency could be switched immediately from 36 GHz to 37.7 GHz. The Sun-new Moon ratios at the two frequencies were found to be 42.6 ± 0.5 at 36 GHz, and 40.6 ± 0.7 at 37.7 GHz.

Using the values for T_M given by Reber³

$$T_s \text{ at } 36 \text{ GHz} = 186 \text{ K} \times 42.6 = 7,920 \pm 90 \text{ K},$$

$$T_s \text{ at } 37.75 \text{ GHz} = 185 \text{ K} \times 40.6 = 7,510 \pm 130 \text{ K}$$

The absolute values of T_s are, of course, subject to the same uncertainties as the absolute value of T_M . The ratio of the two solar temperatures, however, does not have this uncertainty since T_M is nearly constant over the frequency range.

The slope, S , of the brightness curve between two frequencies f_1 and f_2 can be defined as

$$S = \frac{\Delta T_s}{T_s} \frac{f}{\Delta f} = \left\{ 1 - \frac{(T_s/T_M)_{f_2} (T_M)_{f_2}}{(T_s/T_M)_{f_1} (T_M)_{f_1}} \right\} \frac{f_1}{(f_2 - f_1)} \quad (2)$$

where the (T_s/T_M) ratios are determined from equation (1). This definition of slope is the same as the geometric slope of a log-log curve and allows the ratios of the quantities to be used. Substituting the previous values of T_s/T_M for 36.0 GHz and 37.75 GHz into equation (2) gives

$$S = \left(1 - \frac{40.6}{42.6} \times \frac{185}{186} \right) \left(\frac{36}{37.75 - 36} \right) = 1.07 \pm 0.3$$

This result is shown in Fig. 1. Table 1 summarises the slope calculated here, the slope from the van de Hulst model and the slope from the Zheleznyakov model, and gives an estimate of the average slope of the observed disk temperatures from 20 GHz to 40 GHz obtained from Fig. 1.

Table 1 Values obtained for S , the slope of the brightness curve between f_1 and f_2

Observed points 20–40 GHz	$S = 1.4$
Our results	$S = 1.1 \pm 0.3$
Zheleznyakov model ⁵	$S = 1.4$
van de Hulst model ⁴	$S = 0.18$

The steep slope measured at 36 GHz (Table 1) indicates that the observed disk temperatures in the 20–40 GHz range are probably more reliable than the van de Hulst model⁴ calculations. In the centimetre region the observed disk temperatures again join the van de Hulst curve (Fig. 1). There may be a region around 15 GHz where the slope becomes zero, and one would expect no limb brightening to occur. A measurement of the slope between 40 GHz and 50 GHz would determine how the curve blends in with the nearly flat slope found by Reber³ at 50 GHz.

We suggest the future measurements of the solar brightness temperature at millimetre wavelengths should include a measurement of the new Moon. The new Moon serves as a stable calibration source outside the Earth's atmosphere, so that observations of the Sun made with different instruments at different frequencies can be directly compared by considering their Sun to new Moon ratios.

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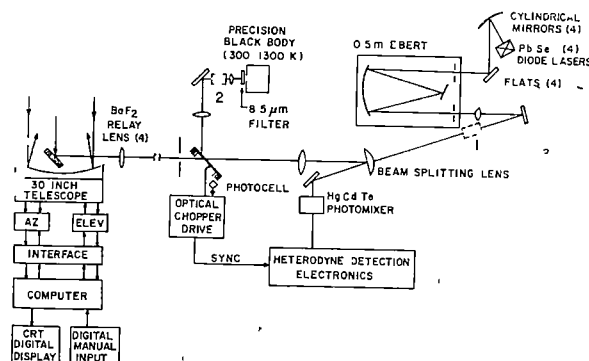
Infrared heterodyne spectroscopy of astronomical and laboratory sources at 8.5 μ

THE first successful infrared heterodyne spectrometer featuring semi-tunable semiconductor diode lasers was constructed and used near 8.5 μ m to make laboratory measurements of line profiles in N_2O and to detect thermal emission from Mars as seen from the Moon. This experiment was conducted at the coude focus of the 30-inch telescope at the Goddard Optical Research Facility in January and February 1974.

In heterodyne detection, infrared radiation (possibly from a remote source) is mixed with the output of an intense coherent local oscillator and a signal is detected at the difference frequency (called the intermediate frequency or if). The spectral characteristics of the remote source are preserved at the if except that the frequency scale is effectively translated by an amount equal to the local oscillator frequency. Using the very best available infrared detectors, and preamplifiers, the bandwidth can extend from close to 'direct current' to above 1 GHz and radio detection techniques may then be used to determine the fine structure of the spectrum of the remote source. The limiting spectral resolution is set by the spread of the local oscillator frequency which for semiconductor diode lasers can be less than 10^5 Hz (ref. 1). Thus, spectral resolutions exceeding 10^8 are possible, far in excess of the resolutions attainable with conventional infrared spectroscopic techniques. Heterodyne spectroscopy is ideally suited for the identification of atomic and molecular species in remote infrared sources and for determination of the line profiles, giving information on the kinetic energy distributions, turbulence conditions, and Doppler velocities of the sources. The extremely high spectral and spatial resolutions make possible the study of low density, low kinetic temperature astronomical sources such as stars, comets, interstellar clouds and the upper atmospheres of planets.

Heterodyne radiometric (non-spectroscopic) methods using gas lasers as local oscillators have recently been successfully applied to detection of broad-band thermal radiation from astronomical sources²⁻⁷ and to laboratory detection of pollutant gases⁸. Although these results demonstrated the feasibility of making such measurements, the real power of heterodyne detection lies in remote spectroscopic measurements at high resolution, long wavelength, and high sensitivity. Peterson *et al.*^{9,10} made the first heterodyne spectroscopic measurements of $C^{18}O_2$ absorption lines in the Mars atmosphere near 11 μ m (see also ref. 10). Their device featured a CO_2 local oscillator and so their observations were limited to the intermediate frequency bandwidth (~ 1 GHz) on either side of the discrete local oscillator frequencies. The versatility of a heterodyne spectrometer is limited by the frequency range over which the laser local oscillator may be tuned and still have sufficient power and coherence for heterodyne operation. Continuously tuneable

Fig. 1 Spectrometer (8.5 μ m) optical system



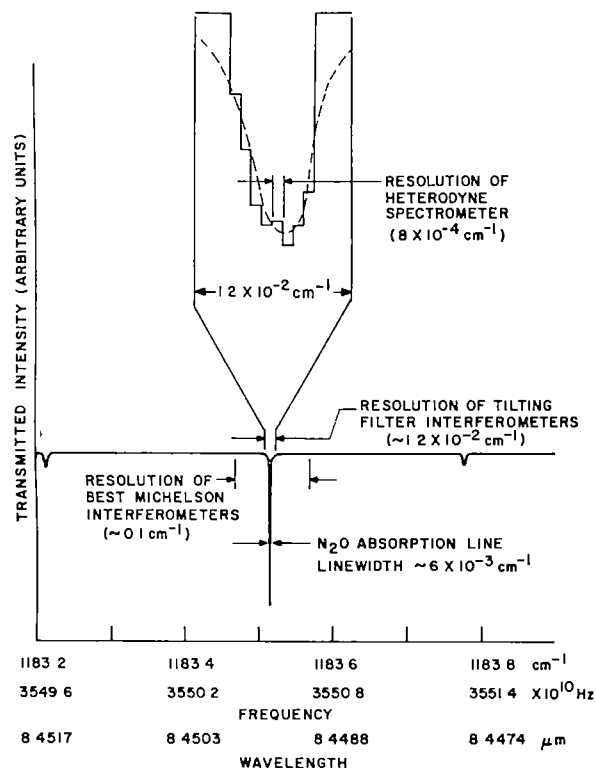


Fig 2 Comparison of spectral resolution of the heterodyne with other high resolution techniques near 8.5 μm

(over several hundred wave numbers) lasers are not yet commercially available in the middle infrared, but semi-tunable lasers have recently become available.

Cryogenically cooled semi-tunable semiconductor diode lasers^{1,11} have been proposed as possible local oscillators for remote heterodyne measurements of air pollutants by Hinkley and Kelley¹². Diode lasers emitting at nominal wavelengths from 5–34 μm can now be manufactured¹³ and are commercially available (from A. D. Little, Inc.). The nominal wavelength can be preselected by varying the chemical composition of the ternary semiconductor compounds. These lasers generally show multimode output, with mode separations of up to several wave numbers. Each mode can be current tuned continuously over $\sim 1 \text{ cm}^{-1}$ ($\sim 3 \times 10^{10} \text{ Hz}$ at 8.5 μm). Thus, tuning is truly continuous over each single mode and piece-wise continuous over the wave length range of multi-mode operation.

We have built a heterodyne spectrometer using PbSe semiconductor diode lasers as local oscillators, a HgCdTe photodiode as a photomixer¹⁴, and an 8-channel filter bank as a line receiver. The spectrometer was interfaced with a computer controlled 30-inch telescope, and a Dicke type chopper was used to look alternately at the astronomical source and a precision blackbody reference source (Fig 1). An array of four current-tuned diode lasers provided adequate local oscillator power for direct absorption measurements over nearly the entire 1,160 to 1,190 cm^{-1} region, however, the range over which the power was sufficient for heterodyne detection was considerably smaller ($\sim 5 \text{ cm}^{-1}$). The maximum total power output from our best diode was $\sim 2 \text{ mW}$ of which $\sim 100 \text{ μW}$ of single mode coherent power was actually incident on our photomixer.

For heterodyne detection the laser and source signals were superimposed by the beam-splitting lens and focused on to the photomixer (Fig 1). A 200-MHz band at the intermediate frequency output of the photodiode was then fed into the eight-channel filter bank and the output voltage from each 25 MHz channel was linearly converted to a frequency which was counted with a multichannel analyser for the period of integration. Data acquisition was synchronised with the chopping frequency. The measured 1 f noise-equivalent-power (NEP) for our system was $1.5 \times 10^{-19} \text{ W Hz}^{-1}$ at 10.6 μm and 100 μW

local oscillator power. At 8.5 μm and 100 μW the extrapolated NEP is $3.6 \times 10^{-19} \text{ W Hz}^{-1}$. This is a factor of two higher than the theoretical limit $h\nu/\eta$, where η = quantum efficiency) and about a factor of two below the result derived from blackbody measurements at 8.5 μm. Neglecting optical losses and taking into account the marginal laser power, the minimum detectable power for our system for 8-min integration times was $\sim 1 \times 10^{-16} \text{ W}$ in a bandwidth of 25 MHz. The resolving power of our heterodyne spectrometer was 1.4×10^6 .

A comparison of the resolution of our heterodyne spectrometer with other high resolution spectroscopic techniques used to measure remote sources near 8.5 μm is given in Fig 2. A portion of the N_2O spectrum near 8.5 μm is shown along with the resolving bandwidth of the Michelson, tilting filter and heterodyne techniques. Only the heterodyne technique is capable

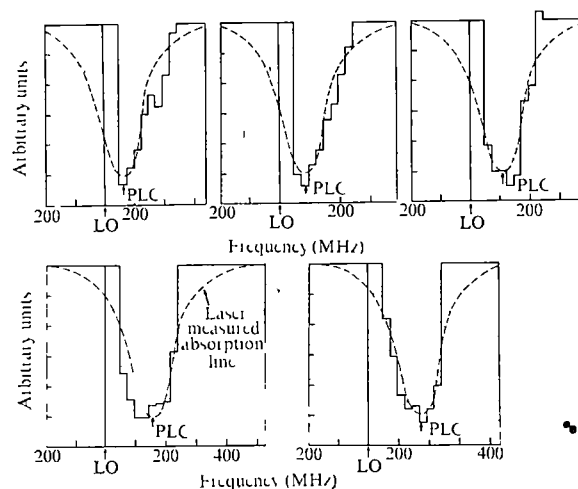


Fig 3 Heterodyne detection of N_2O absorption line ---, Absorption line profile measured in the direct detection mode. The histogram corresponds to the line profile measured in the heterodyne mode in the eight-channel filter bank.

of line profile measurements of the N_2O lines. Line profiles in the ν_1 band (100–000) of N_2O were measured directly and in the heterodyne mode (Fig 3).

A cell filled with N_2O at 10 torr pressure was placed in position 1 (Fig 1) and the absorption line positions and profiles were determined by direct detection of tuned laser line absorption. The cell was then placed in position 2 and the lines were heterodyne detected in absorption against a 1,300 K blackbody continuum (the optical path to the telescope was blocked). The line was then moved through our filter bank by tuning our local oscillator slightly between measurements. The pressure broadened absorption linewidth was about 170 MHz. For comparison purposes, both lines were normalised to the same amplitude and the direct detected line was drawn at the predicted line centre (PLC) position in the filter bank. The noise is greater in the heterodyne line profile because the blackbody power in a 25 MHz heterodyne channel corresponds to $\sim 10^{-13} \text{ W}$ ($\sim 10^{-14} \text{ W}$ at line centre) whereas the direct measurements correspond to $\sim 10^{-4} \text{ W}$ of laser power at 8.5 μm. We believe that this is the first time that a molecular absorption line profile has been measured using both active and passive measurement techniques.

The heterodyne system was next applied to astronomical observations and was used to measure spectroscopically thermal radiation from Mars and from the Moon (Fig 4). The blackbody continuum near 8.5 μm gave heterodyne signal-to-noise ratios of 3 to 8. The variations were caused by daily changes in the local oscillator power and changing atmospheric conditions. The theoretical system signal-to-noise ratio for a blackbody source is given by^{6,15} where B is the 1 f bandwidth,

$$S/N = \alpha \eta_{\text{eff}} (B \tau^{1/2} / (\exp(h\nu/kT) + 1)) \quad (1)$$

τ the integration time and α the transmission of the atmosphere and optics, η_{eff} is the effective quantum efficiency of our photodiode and is equal to the true quantum efficiency degraded by a factor stemming from the lack of sufficient local oscillator power

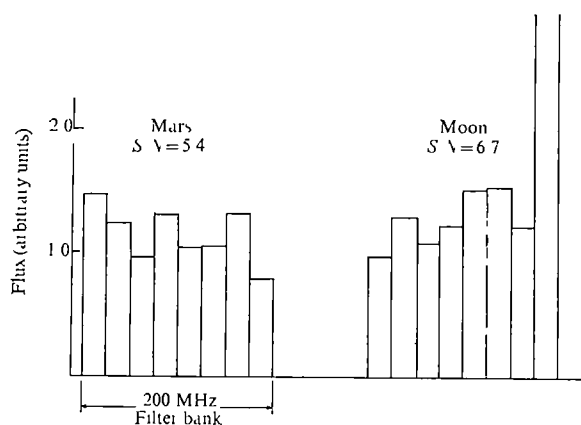


Fig 4 Heterodyne signals at 8.5 μm from the Moon and Mars. The signal in each channel is represented by a bar whose height is proportional to the net flux received from the source. The huge signal in the last channel of the lunar data was probably a system noise pulse and was disregarded in determining the signal-to-noise ratio

to reach the shot-noise limit. The true quantum efficiency of our photodiode at 8.5 μm was about 13% and η_{eff} was estimated to lie between 2% and 65% depending on laser operation.

Theoretical signal-to-noise ratios (with $\alpha = 1$ in equation 1) based on local lunar temperature at the region of observations, known electronic parameters, and the appropriate η_{eff} were from 5 to 10 times greater than the experimentally obtained values, implying $\alpha = 0.1 \rightarrow 0.2$. Optical transmission measurements gave $\alpha \approx 0.16$, which is consistent with the range of values obtained by comparing the experimental and theoretical S/N ratios discussed above.

The observed S/N ratios for Mars are more compatible with a temperature of 300 K than the expected value of $\sim 250^\circ\text{K}$ (refs 9, 10 and V. Kunde, private communication). The effective quantum efficiency changed between the measurements of Moon and Mars because of the greater laser output during the Mars run. The quantitative results are of only limited accuracy because of the frequent variation in laser power, poor telescope pointing stability, changes in zenith angle between runs and daily changes in atmospheric conditions. Accurate monitoring of all these parameters was not feasible at the time of the experiments.

The blackbody power from Mars incident on the heterodyne detector in a bandwidth of 25 MHz is $7 \times 10^{-16}\text{ W}$. Conventional techniques are more sensitive for broad-band measurements of blackbody radiation, but spatially localised, narrow spectral lines (with linewidths $\geq 100\text{ MHz}$, say) containing an equivalent energy would be undetectable using conventional techniques. The line profiles most certainly could not be observed. Although our astronomical measurements were not of spectral lines the power detected was of the order expected from remote line sources. The high resolution of our tuneable heterodyne spectrometer (as shown in the laboratory spectral line study) together with its high sensitivity (as demonstrated in the astronomical measurements) make it a suitable instrument for detecting and mapping narrow low intensity spectral lines over a broad wavelength range in the infrared.

Although we were able to obtain heterodyne signals with diode lasers as local oscillators, the measurements were far from straightforward. The coherent output power from presently available diode lasers is generally below that required for shot noise limited heterodyne operation. Because of the characteristics of the diode lasers, great difficulties were encountered in the design of the optical system to collect, focus and match the laser output to our heterodyne field of view. These problems can be eliminated by using better diode lasers and some success has already been achieved in growing more powerful lasers (see, for example, ref 16). The output of the diode laser was sensitive to nearly all environmental effects such as room temperature variations, mechanical and acoustic vibrations, the liquid helium level and the heat sinking parameters of the helium Dewar. The high

resolution of our instrument requires stabilities of better than 0.6 ppm in the focusing optics and the parameters of the diode laser. Meaningful measurements could only be made when the system remained stable during the period of data acquisition. Needless to say, conditions sometimes changed between measurements, making accurate comparisons between runs difficult.

These results demonstrate the capabilities of a heterodyne spectroscopic system and that heterodyning can be achieved using semiconductor diode laser local oscillators. With improved design and better diode lasers future systems should approach the stage at which their operation is limited only by quantum noise.

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Origin of magnetite and pyrrhotite in carbonaceous chondrites

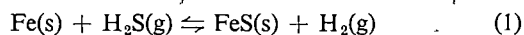
CARBONACEOUS chondrites, although comprising only about 2% of known meteorites, are extremely interesting for scientific investigation. Their mineral constitution, and the correspondence between their bulk chemical composition and the solar abundance of condensable elements, indicate that minimum chemical fractionation and thermal alteration have occurred. The mineral phases observed in these primitive chondrites are sufficiently unique, with respect to other meteorite classes, to have elicited considerable speculation about the physical environment in which they formed¹⁻⁷.

We consider here two minerals—magnetite and pyrrhotite—present in some carbonaceous chondrites and propose that a substantial fraction of the magnetite, at least, resulted from the

oxidation of troilite. Pyrrhotite is expected as a direct consequence of magnetite formation through this reaction. Previously, meteoritic magnetite was considered to have resulted from the oxidation of metallic iron⁸ or from the alteration of olivine⁹. No adequate genesis has been proposed for pyrrhotite¹⁰.

The formation of magnetite from troilite has been observed¹¹ during thermomagnetic analyses—measurement of saturation magnetisation against temperature—on meteoritic troilite. We observed similar behaviour during similar experiments¹² on carbonaceous chondrites and troilite separates from the Staunton iron meteorite, both under vacuum and in a fugacity-controlled atmosphere¹³. As magnetite was observed to form under vacuum, it is evident that troilite reacts with gaseous oxygen, rather than with the gases used for fugacity control. The fact that magnetite formed from troilite on a time scale of minutes prompted us to inquire whether meteoritic magnetite may have formed similarly.

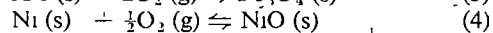
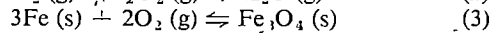
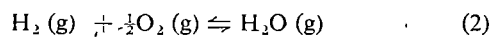
Urey⁸ concluded from thermodynamic arguments that, in an atmosphere with a solar composition of H_2 and H_2S , reaction (1) would proceed to the right below about 700 K. On that basis, meteoritic troilite is usually considered a stable phase below that temperature. The inferred 'stability', however, is relevant only with respect to H_2 and H_2S , to the exclusion of other gases and, therefore, yields no information on the reaction of troilite with O_2 .



Despite the ubiquitous presence of troilite in chondritic meteorites¹⁻⁷, few studies have been made of its stability and the possibility of its oxidation. Above 413 K, troilite converts to hexagonal low-temperature pyrrhotite¹⁴. Taylor¹⁵ has determined the approximate path that pyrrhotite (and presumably troilite) takes on oxidation in air. During oxidation, the sulphide mineral becomes increasingly iron deficient, with the eventual formation of the end member of the oxidation series, pyrite. Apparently, the effective fugacity of sulphur increases (without the addition of that element) whereas the iron activity decreases, the iron being partitioned into the oxide phase.

During thermomagnetic experiments on troilite (some conducted at constant temperature), we observed magnetite formation at temperatures as low as 373 K, provided that the oxygen fugacity was held in the magnetite stability field, and that the troilite was sufficiently finely divided. As expected for a grain-surface reaction, the rate and extent of reaction were strongly dependent on the grain size. Furthermore, we have observed thermomagnetically the formation of pyrrhotite, which confirms Taylor's work¹⁵.

If meteoritic magnetite resulted from the oxidation of troilite then the reaction must have been able to occur under conditions expected in the early Solar System. The petrological evidence must also be compatible with the expected consequences of magnetite formation by that means. Therefore, consider the oxygen fugacity equilibria for three reactions



Since the oxygen fugacity in an atmosphere of solar composition is governed by reaction (2), the oxygen fugacity can be calculated as a function of temperature

$$-\log_{10}(f_{O_2}) = (-2 \Delta G_f^\circ / 2.3RT) - 2 \log_{10}(H_2O/H_2) \quad (5)$$

where the ratio H_2O/H_2 is determined from cosmic abundances¹⁶ and ΔG_f° is taken from thermodynamic data. Oxygen fugacity equilibria for reactions (3) and (4) can also be calculated

$$-\log_{10}(f_{O_2}) = -\Delta G_f^\circ / 2.3nRT \quad (6)$$

using the appropriate thermodynamic data and noting that for

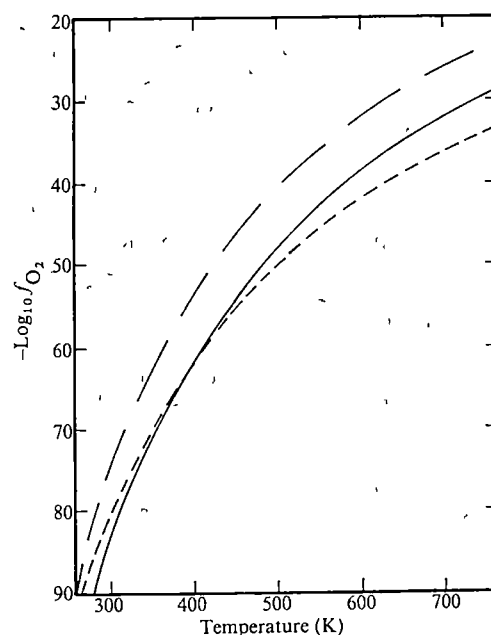


Fig 1 Stability fields of Ni-NiO, Fe-Fe₃O₄ oxygen fugacity and the equilibrium oxygen fugacity expected in an atmosphere with a cosmic abundance of H_2 and H_2O ———, Equilibria of Ni-NiO oxygen fugacity. The NiO stability field lies above this curve, metallic Ni below ———, Equilibria of Fe-Fe₃O₄ oxygen fugacity. The magnetic stability field lies above this line, metallic iron below ———, Oxygen fugacity in an atmosphere with a solar abundance of H_2 and H_2O .

equations (3) and (4), $n = 2$ and $n = \frac{1}{2}$, respectively. For temperatures under consideration here, FeO equilibria can be ignored.

The results of these calculations are shown in Fig 1. Below about 400 K the oxygen fugacity expected in an atmosphere with a solar composition of H_2 and H_2O lies within the magnetic stability field. These calculations demonstrate that provided that equilibrium was maintained, magnetite formation from condensed troilite is possible at temperatures at which we have determined that troilite can readily undergo oxidation. The oxygen fugacity expected in the early Solar System lies well within the metallic nickel stability field at all temperatures. Nickel oxide would not be expected, and any nickel present in the condensate must have existed in a metallic state or in some other chemical form, such as a sulphide.

Next consider the oxidation of meteoritic troilite under conditions expected in the early Solar System. As oxidation progresses, the sulphide mineral becomes iron deficient. Any nickel in the troilite should not oxidise at the oxygen fugacities expected, and, therefore, would remain in the sulphide phase. Thus, the sulphide mineral would become increasingly rich in nickel and increasingly deficient in iron. The degree to which sulphur is partitioned between the sulphide mineral and the gaseous sulphur phase may be a function of the prevailing sulphur fugacity in the system, and is difficult to assess. In a fully condensed system heated as high as about 400 K, the sulphur pressure will depend on whether the system is closed—as expected for subsurface regions of a planetoid—or open (even partially open)—as expected for small porous bodies, for near surface regions of the planetoid, or for material dispersed in space. In an incompletely condensed system, additional complications may arise as a result of sulphur present in the vapour phase. Nonetheless, it is possible to conceive of a fully condensed system subjected to mild heating and open to the loss of the gaseous sulphur phase. Such a system closely approximates the conditions existing during our experimental studies.

If meteoritic magnetite formed from troilite oxidation, as we

suggest, several consequences may be expected (and are observed) in meteorites. From the chemical arguments, discussed here, we expect that any magnetite formed from troilite would contain little nickel, even if the oxidation had been extensive. Magnetite in carbonaceous chondrites has an extremely low Ni content^{13,17}

We also expect the sulphide minerals resulting from troilite oxidation to attest to the degree of oxidation, thus, large grains, subjected to mild oxidation, should form pyrrhotite, somewhat enriched in Ni. Pyrrhotite has been observed in the Orgueil¹⁸ and Revelstoke¹⁹ meteorites, both of which contain abundant magnetite 11.9% and 7.2% respectively¹³. The Ni content of the pyrrhotite phase of Orgueil (~1.0%) is significantly higher than that of meteoritic troilite (~0.2%). Unless an alternative mechanism is found, it seems that the observed pyrrhotite resulted from the oxidation of troilite, which verifies our thesis. Furthermore, extreme oxidation of troilite is expected to result in pyrite formation. The only meteorite in which pyrite has been observed²⁰ is the 'recrystallised' carbonaceous chondrite Karoonda using thermomagnetic measurements we found that it also contains abundant magnetite (7.7%). Finally, the oxidation of troilite may yield a source of SO₂ for the formation of soluble sulphate species observed in carbonaceous chondrites¹. If so, the sulphur in the sulphate phase should be isotopically lighter than the sulphur remaining in the sulphide phase. For Orgueil, at least, that is the case²¹.

We propose that much (if not all) of the magnetite observed in carbonaceous chondrites resulted from troilite oxidation at temperatures ≤ 400 K and that pyrrhotite formation is an expected consequence of this reaction. We have observed experimentally the oxidation of troilite over a time scale ranging from a few minutes to a few hours (depending on grain size and temperature) under conditions similar to those expected in the early Solar System. It seems that the previously inferred 'stability' of troilite in an atmosphere of solar composition, based on reaction (1), is invalidated by the very real possibility of reaction with oxygen. We suggest that further experimental work on the oxidation of troilite, specifically regarding the influence of sulphur fugacity, may yield data useful in establishing constraints on permissible modes of major element condensation and on metamorphic conditions present during the formation of chondritic meteorites.

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Detailed structure of a mantle seismic zone using the homogeneous station method

As the accuracy of location of mantle earthquakes has increased, the suggested thickness of seismic zones within the mantle has decreased¹ and special studies are now needed to determine the detailed structure of these zones. In this study we used the homogeneous station method to locate earthquakes occurring at intermediate depths beneath the North Island of New Zealand, and here report that the hypocentres could lie on two dipping surfaces about 9 km apart. Using the plate tectonics hypothesis these surfaces may be related to the top and bottom of a dipping, brittle zone, either down-going oceanic crust^{2,3}, or some colder region within the descending lithosphere⁴.

The intermediate zone under North Island strikes approximately north-west, dips at about 67° to the horizontal and is overlain by the stations of the seismic network run by the Seismological Observatory at Wellington. From the Observatory's readings for the period November 1965-December 1972 (excepting 1970, when readings were unavailable), all subcrustal earthquakes were selected which had P or P-wave phases recorded at seven selected seismic stations (see Fig. 1) and had all but one of these phases recorded to the nearest tenth of a second, the others were recorded to the nearest second. The 73 earthquakes selected, which included 26 with one reading to the nearest second, were relocated using the standard least squares method, the seven P-wave readings, and the Jeffreys-Bullen tables.

The homogeneous station method, which requires that each station records the P-wave phase of each earthquake, is the simplest of the more accurate relative hypocentre location procedures (paper in preparation). The relative mislocation of a pair of events will depend only on the reading errors and the change, from one event to the other, in the model travel time errors—these are the discrepancies between the actual travel times from the true hypocentres to the stations and the times given by the travel-time tables used. Confidence ellipsoids for the relative locations were calculated using time residuals. Fifty-four of the hypocentres within, or close to, the edges of the station network were divided into eight geographical groups, the hypocentres within each group being within a radius of 40 km. If r_{ij} is the time residual of the j th station from the i th event in a group, then we have a model

$$r_{ij} = S_j + \epsilon_{ij}$$

where the S_j s are the mean station residuals for the group and are related to the mean model travel time errors, and the ϵ_{ij} s are related to the reading errors and the variation of the model travel-time errors about their means. We assume the quantities ϵ_{ij} have independent normal distributions with zero mean and a variance σ^2 . The station terms S_j for each group were determined (see Table 1) and a pooled estimate of σ^2 was made for all groups. This gave an estimate of 0.39 s for σ , based on 114 degrees of freedom (54 × 7 residuals - 54 × 4 hypocentral parameters - 8 × 6 station terms). Thus, the 90% confidence ellipses are given by

$$(x, y) A_g^{-1} = 2(0.39)^2 F(2, 114) \quad (1)$$

where A is the appropriate 2 × 2 submatrix of the inverse of the condition matrix of the least squares equations and $F(2, 114)$ is the 90% value of the F distribution.

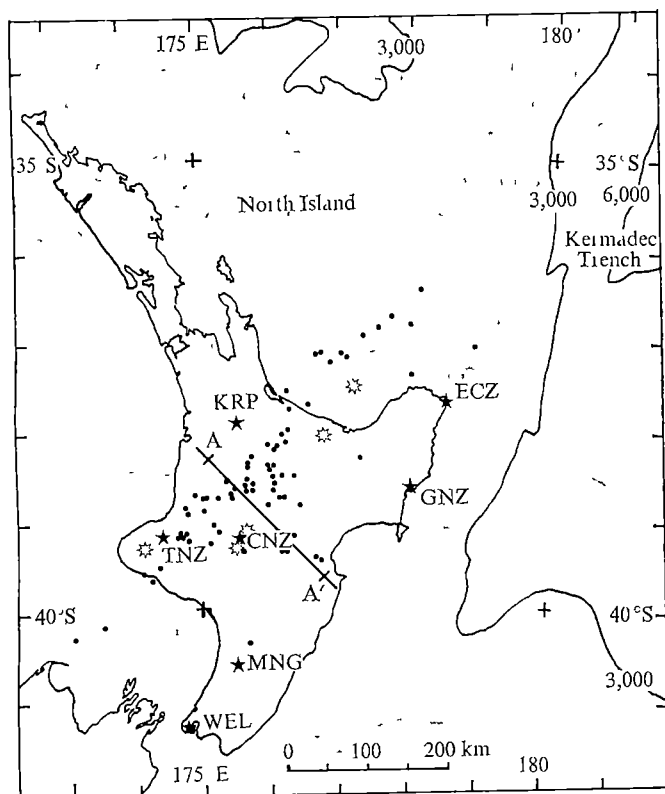


Fig 1 Map and bathymetry of North Island. ●, Epicentres of the earthquakes considered, solid stars, seismic stations used, open stars, active andesite volcanoes. Solid line, depth contours showing depth in metres.

A plane with strike N 45 25° E and dip 67 25° was fitted by the least squares method to all but one of the hypocentres which fall within the net and which were deeper than 118 km. The sum of squares of the perpendicular distances of these 49 hypocentres from the plane was minimised and the standard deviation of the distances was 5.6 km. The Seismological Observatory's hypocentres for the same earthquakes were distributed about a similar plane with a standard deviation of 10.0 km. Hamilton and Gale⁶ fitted median curves to Observatory hypocentres in the same area, and found standard deviations of about 13 km. Clearly, the homogeneous station method locates the hypocentres much more tightly. Typical 90% confidence ellipses in a vertical plane perpendicular to

Table 1 Residuals, stations means, sums of squares about these means, and hypocentre standard errors for one of the geographical groups of earthquakes considered in the text

	CNZ	ECZ	GNZ	KRP	MNG	TNZ	WEL	Standard error
Residuals								
	0.3	0.5	-0.1	-1.1	-0.2	1.2	-0.7	1.3
	0.3	0.5	-0.4	-0.7	0.2	0.7	-0.6	1.0
	0.1	0.5	-0.1	-0.9	0.1	1.2	-0.9	1.3
	-0.1	0.2	0.1	-0.7	0.2	1.1	-0.8	1.2
	0.1	-0.5	0.0	-1.0	0.0	1.3	-0.9	1.4
	-0.4	-0.2	0.4	-0.3	0.3	0.7	-0.6	0.8
	-0.3	-0.1	0.4	-0.6	0.2	1.1	-0.8	1.1
	0.0	-0.2	0.3	-1.0	-0.3	1.4	-0.7	1.4
Mean	0.00	0.26	0.08	-0.79	0.06	1.09	-0.75	
Sum of squares	0.46	0.59	0.55	0.46	0.32	0.43	0.10	

For location of seismic stations see Fig 1

our plane, and given by equation (1), are shown in Fig 2. The average standard deviation in the direction perpendicular to the plane, based on the estimate of 0.39 for σ , is 3.5 km. Thus, we have good accuracy perpendicular to the zone below about 120 km but not above that depth, where the zone becomes more horizontal. (The earthquake below 118 km excluded from the analysis is isolated and its epicentre is about 38° 3' S, 117° 2' E. Various depth determinations in kilometres are ours 160, the Seismological Observatory's 189, US Coast and Geodetic Survey 114 and International Seismological Centre 100.)

Using the plane of best fit as a reference plane a quadratic surface was fitted to the 49 hypocentres by minimising the sums of the squares of distances from the hypocentres to the surface in the direction perpendicular to the plane. The standard deviation, 4.2 km, was significantly better than that for the plane, but more importantly it was noticed that although most of the hypocentres lay close to the surface, 10 widely spread

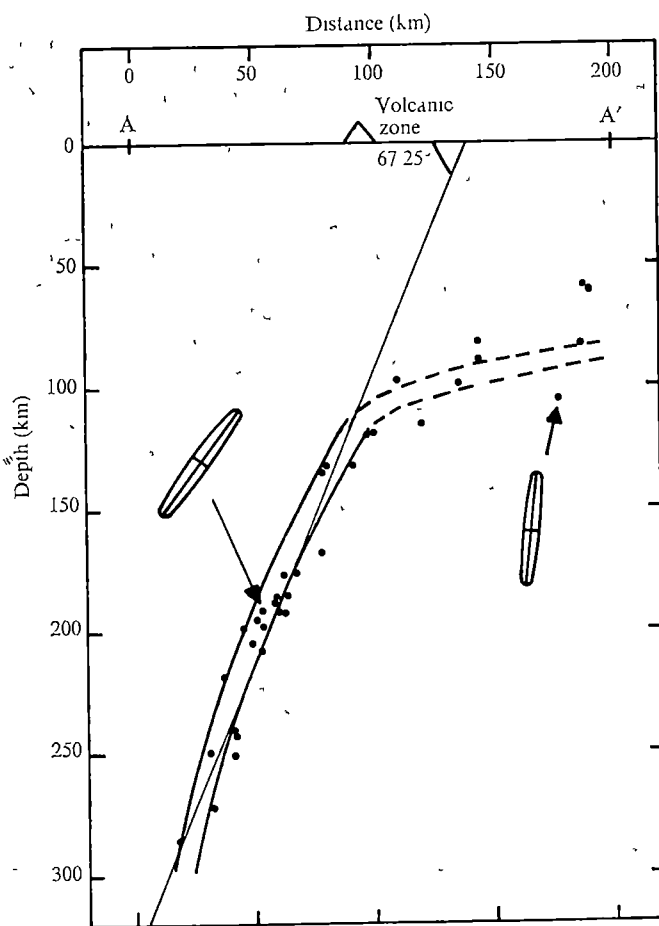


Fig 2 Cross section of the seismic zone through AA' (Fig 1), showing hypocentres (●) within 50 km of the section, typical 90% confidence ellipses of relative locations and sections through the best fit plane —, The quadratic surfaces 9 km apart (described in the text), ---, possible extensions of these surfaces

hypocentres were significantly above it in a direction perpendicular to the plane. A quadratic surface was fitted to the larger group of 39 earthquakes and the standard deviation of hypocentres about this surface was 3.4 km—remarkably close to the value of 3.5 km obtained independently from the residual analysis. The remaining 10 hypocentres had a mean displacement of 8.8 km from the surface (perpendicular to the plane). The standard deviation about their mean was 1.5 km, but this low figure probably results from the biased sampling technique. The histogram of distances from the surface is shown in

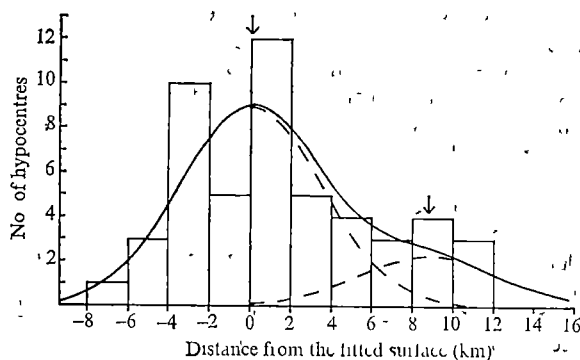


Fig. 3 Histogram of distances of hypocentres from the lower quadratic surface (see text), and the predicted distribution of 39 hypocentres on the surface of the plane and 10 hypocentres 8.8 km above the surface of the plane, with normal distributions and standard deviations about their means of 3.5 km

Fig. 3 together with the predicted distribution for 39 hypocentres on the surface of the plane, and 10 hypocentres 8.8 km above the surface of the plane, with normal distributions and standard deviations of 3.5 km about the means in both cases. The evidence suggests strongly that the North Island deep seismic zone is about 9 km thick and that the earthquakes initiate in the top and bottom surfaces of the zone. Mechanism studies⁶ show no clear differences between earthquakes originating on either surface, and our results differ, therefore, from those from the Kurile Islands⁷ where there is a 30 km thick zone with differing earthquake mechanisms above and below. Further studies are to be done to locate less well recorded earthquakes and to improve the absolute locations.

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Possible large creep event apparently preceded by a dilatant precursor

THE invar wire earth strainmeter at the Nelson underground field station of the Physics and Engineering Laboratory, New Zealand, has produced the record shown in Fig. 1. The size of the two strain steps shown was far too great to be recorded on the tidal chart recorder but have been established with complete confidence from the amount of mechanical adjustment needed at the time of the inspection visits. Long term invar expansion has been removed from the curve shown. This is measured independently on an identical instrument decoupled from the earth using a sample of the same wire held at the same temperature and tension, but referred to very old large diameter Pyrex glass tubes as a reference length. Ideally, these should be of fused silica. The equation of the long term expansion agrees quite closely with that reported by Kaye¹ in 1911.

There were no earthquakes at the times of the two events shown and it is postulated that the strain change of 1.2×10^{-4} was produced by a large creep of about 20 cm, possibly on the nearby Waimea fault. On the surface this lies 5 km from the station but the creep was perhaps deep and without surface expression. In contrast to California, most seismic events in New Zealand do not correlate well with known active faults² and this event may be no exception. The apparent precursor about 20 months before would, if caused by rock dilatancy, indicate an earthquake of about magnitude 6 (ref. 3), but instead an aseismic creep event occurred. Work by Chinnery⁴ indicates that a 20 cm displacement is of the order to be expected from an earthquake of magnitude 6. The second much smaller event is regarded as an 'after-creep' of the first.

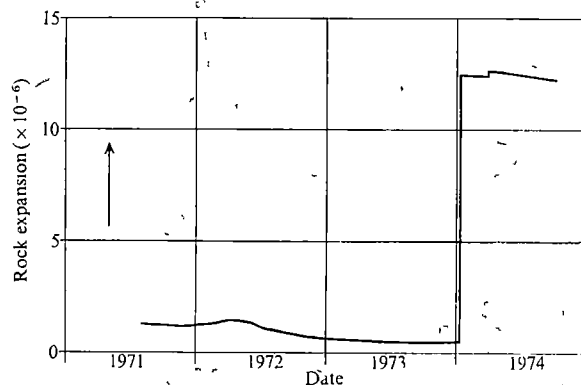


Fig. 1 Long term earth strain record from Nelson underground field station (41° 5' S, 172° 9' E, 150 m deep) from the time recording began in July 1971. The installation of the instrument⁶ was carried out in early February 1971. The curve has been derived both from measurements at the time of the inspection visits and from the high sensitivity chart recorder which has an automatic stepper giving an effective range of approximately 70 chart widths.

Wire tension increased at the time of each event and therefore they can hardly be attributed to instrumental defects. No other comparable events have been recorded on two other strainmeters of the same design which have been operating at other distant stations for similar periods of time. There is also a difference in the rate of strain accumulation before and after the event. Extrapolation of the present rate would indicate another such event in approximately 20-25 yr which, of course, might then appear as an earthquake. It is interesting that in 1941 there was an earthquake of magnitude 5.5 at just this location⁵ (41° 6' S, 172° 9' E), although at a depth of 80 km.

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Mechanism for the persistence of tectonic lineaments

It is widely recognised that major faults and other linear zones of tectonic activity may remain intermittently active through extended periods of geological time. I suggest a mechanism which may account for this persistence.

Examples of long lived linear tectonic belts are known in most continents although their significance was probably

first appreciated in the Soviet Union where activity on the deep faults bordering the southern margin of the Siberian shield is known to extend from Archaean to late Phanerozoic times¹⁻³. In Africa the present rift zone has been identified as the locus of Precambrian activity⁴, and Precambrian lineaments in the West African and Guyana shields were reactivated during the Mesozoic separation of Africa and South America⁵.

Two of the major Precambrian boundaries in Greenland have been shown to have remained active for up to 1,000 Myr from their initiation in the Archaean⁶, and more recent work on the Nagssugtoqidian boundary has extended the probable period of intermittent activity to 2,500 Myr. The earliest recognised activity at this boundary is large scale ductile transcurrent movement along⁷ a vertical shear belt, 35 to 40 km wide, along which movement began about 2,650 Myr ago. Following further activity in the Precambrian, the boundary region was the locus of intrusion of a suite of kimberlite and associated dykes of Cambrian age⁷. The suggestion that movements continued during the Phanerozoic⁸, based on the possibility of pseudotachylites cutting dykes of the kimberlite suite, has since been confirmed (J. Grocott, personal communication). Offshore seismic work in connection with hydrocarbon prospecting has shown faulting or fault controlled deposition of Mesozoic sediments along the line of the Precambrian shear zone. This not only confirms that the lineament has remained intermittently active for more than half of the Earth's history, but also illustrates the economic significance of such lineaments.

Any explanation for the persistence of activity along a major linear movement zone is subject to two principal constraints. The persistence of activity must be provoked within the lithosphere because, over the time scale considered, no part of the lithosphere is likely to have remained fixed in position relative to underlying mantle. On the other hand, translation of plates and displacements within them result from causes external to the lithosphere. Both these requirements are met if a lineament is the expression of a persisting mechanical weakness, along which displacements which otherwise would be more widely distributed have been localised. If that is the case a lineament is likely to accommodate a variety of movement directions during its active life. An appropriate mechanical weakness would be one affecting a major part of the thickness of a lithospheric plate and would not be restricted to the relatively thin upper portion where brittle fracture is dominant in rock failure and displacement.

The significance of large scale zones of ductile simple shear strain has become apparent recently^{9,10}. In particular, it is clear that they are the deeper and mostly aseismic continuations of structures which appear at higher crustal levels as major faults, thrusts, and brittle fracture zones. A characteristic of ductile shear zones, most easily seen in small scale examples up to a few metres wide, is that the deformation, although plastic, is usually accompanied by a reduction in grain size. Theoretical and experimental studies^{11,12} suggest that grain size is a significant variable in determining rates of plastic deformation, because of the predominant role of grain boundary diffusion in deformation at high temperature and low stress. A theoretical model in which strain rate varies in inverse proportion to grain diameter¹³ is in substantial agreement with strain rate experiments on the deformation of marble at high temperature¹⁴. Other theoretical studies indicate the importance of grain size in determining creep rates¹⁵. An acceleration of strain rates is to be expected towards the centre of ductile shear zones where, because of boundary effects, initial strains are highest and the initial reduction in grain size most marked. In a rock mass which is anisotropic in respect of grain size, and hence ductility, the application of deviatoric stresses will produce strain along suitably oriented

existing planes of finer grained and more ductile rock—those zones along which previous ductile displacement took place without subsequent recovery of grain size by grain growth. It is then important to determine whether or not planar zones of finer grained rock, seen at present levels of erosion in Precambrian basement rocks as ductile shear zones, extend through an appreciable thickness of the lithospheric plate. The scale of transcurrent displacements along major lineaments, whether now observed as brittle faults or ductile shears, is commonly of the order of tens or hundreds of kilometres and difficult to account for otherwise than by extension of the displacement downwards as far as the decoupled zone at the base of the lithosphere. The identification of intense tectonic fabrics in upper mantle rocks now seen as inclusions in kimberlites, dykes and pipes is particularly interesting. These fabrics have been particularly well demonstrated in a series of deformed mantle rocks from kimberlites in Lesotho¹⁶. Comparable fabrics exist, although less well developed, in the kimberlites from the Nagssugtoqidian boundary. Such examples, with textures identical with those of deformed crustal rocks, show the similarity of deformation processes at widely separated levels within the lithosphere, and demonstrate that ductility increase by grain size reduction can reasonably be regarded as likely to be effective on the scale of the lithospheric plate. Such weakening can only be responsible for the persistence of lineaments if recovery of grain size by grain growth is not effected in the time interval between successive increments of movement along the lineament. Recovery times are certainly very prolonged in crustal rocks as evidenced by the ubiquity of grain size anisotropy in tectonites, and by the absence of grain growth fabrics in the inclusions from both Lesotho and Greenland kimberlites, which suggests that the rates of recovery of grain size in mantle rocks are also slow relative to strain rates.

So I suggest that the concept of mechanical weakening through reduction in grain size may account for the longevity of major tectonic lineaments, this proposal can be tested by work on the fabrics of crustal and mantle tectonites, and on annealing rates. Future work should also show whether the distribution of tectonised mantle inclusions in kimberlites is related to tectonic lineaments, or whether kimberlite distributions are related to such lineaments as seems to be the case on the Nagssugtoqidian boundary and has been suggested for kimberlites in Southern Africa¹⁷ and Siberia¹⁸.

I emphasise that these proposals are complementary to current plate tectonic theory, plate boundaries initiated in recent geological time (the late Phanerozoic), in several cases seem to coincide with older lineaments.

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The February–June weather relationship in north-west Europe

It has been well said by Bonacina¹ that everything which happens to, or in, the atmosphere, affects its subsequent behaviour. In other words, there is a "memory" in the atmosphere, probably such that an anomaly produced at one time may lead to a similar, or a related, anomaly being restored in the future. It is known that although some factors can be neglected for short range forecasting, they become progressively more important in the longer range. These factors are unknown, although most meteorologists would probably agree with Namias² that two of the most important must be extraterrestrial events, such as variations in solar activity, and variations in the character of the Earth's surface.

But empirical methods of long range forecasting are sometimes found simply by discovering relationships between observations at different times and places, and there seems no reason for not using them in prediction. The physical mechanism behind the relationship may not be fully understood, but the empirical discovery usually points to the research required for understanding the mechanism. Here I illustrate this idea by an example.

In the course of looking at climatic trends in north-west Europe, particularly the northern North Sea area, certain relationships were noted between weekly or monthly periods in the winter season and periods of similar length in the following summer. This may best be illustrated by what one may call the February–June relationship. Figure 1 shows the

Fig 1 February and June mean temperatures, °C, Dalen 1 Telemark, 1940 onwards (Pre-1950 shown by crosses)

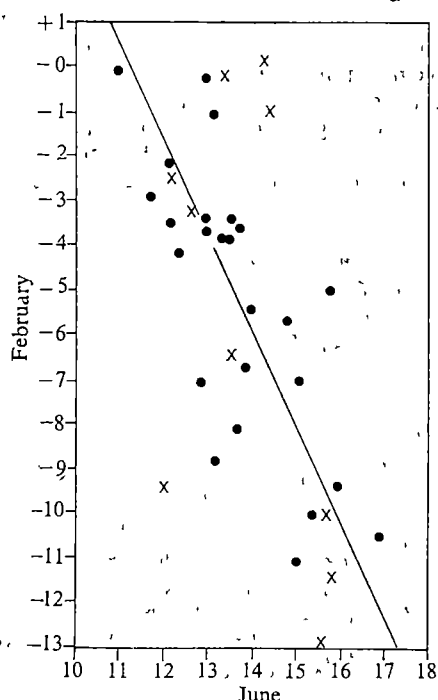


Table 1 Dalen 1 Telemark, mean temperature in June (°C)

	Forecast	Actual	Trend from previous June correct	Forecast within 1° C of actual
			(C)	(C)
1939	—	13.6	—	—
1940	16.5	15.8	C	C
1941	15.9	15.7	C*	C
1942	15.6	12.0	C	—
1943	11.3	13.4	—	—
1944	12.4	12.2	C	C
1945	12.9	13.2	C	C
1946	12.8	12.7	C	C
1947	17.3	15.5	C	—
1948	14.2	13.5	C	C
1949	11.1	14.3	—	—
1950	13.0	13.0	C	C
1951	13.2	12.9	C*	C
1952	13.0	12.6	C*	C
1953	13.8	15.9	C	—
1954	14.3	13.3	C	C
1955	14.4	12.9	—	—
1956	15.0	14.3	C	C
1957	13.3	13.4	C	C
1958	14.8	13.7	C	—
1959	13.8	14.1	C	C
1960	14.4	15.1	C	C
1961	13.3	13.8	C	C
1962	12.8	12.1	C	C
1963	15.0	15.4	C	C
1964	13.2	12.2	C	C
1965	12.9	13.3	C	C
1966	15.6	15.1	C	C
1967	13.2	13.6	C	C
1968	13.9	14.9	C	C
1969	15.1	16.0	C	C
1970	15.5	16.8	—	—
1971	12.5	13.2	C	C
1972	13.4	12.4	—	—
1973	12.5	14.4	C	—
1974	12.6	13.9	C	—

* Almost the same as previous year

relationship between February and June from 1940 onwards for Dalen 1 Telemark (59°27'N, 8°0'E), an inland station in southern Norway. A regression line can be drawn which shows that the mean temperature in February is inversely related to the mean temperature in June. If this regression line is used at the end of February to estimate the mean temperature for the following June, the results compare very favourably with the actual temperatures (Table 1).

It will be seen that in 30 years out of 35 the estimate was correct in the sense that it predicted correctly whether the June mean temperature would be higher or lower than (or very nearly the same as) that of the previous year. In 25 years out of 35 the mean temperature was predicted correctly to within 1.0° C and in 14 of those years it was correct to within 0.5° C. The accuracy of the estimates increased until the past few years, when it has deteriorated slightly. Some variations in this simple predictive method were investigated, mainly to try to take into account year-to-year trends, but this has not led to any general improvement in the method.

Periods other than calendar months were not used because of the greater amount of work involved. Had this been done, it would have rectified some, if not all, of the least successful predictions. For example, the prediction of a very warm June in 1955 proved to be incorrect, but in fact July proved to be exceptionally hot.

This February–June relationship was explored (1) backwards in time, and (2) in neighbouring areas. At Dalen 1 Telemark, during most of the 1940s, the relationship came close to the regression, but this was not the case from 1891 to the 1940s. There was, however, a suggestion of two populations, one with a direct correlation and another with an inverse correlation, the latter applying to all cases where the February mean temperature was -6.0°C or below. Examination of other stations in Norway, Denmark, Scotland and England indicated that the inverse correlation between February and

June mean temperatures had been spreading south and west. There is more scatter about the regression line at coastal stations, but, for practical purposes this is compensated for by the angle of slope of the regression line approaching 45° .

In both Norway and Scotland, higher than average temperatures in winter correlate well with higher than average rainfall, since both occur in westerly and cyclonic conditions. Conversely, there is relatively little precipitation in anticyclonic conditions. In summer there is an inverse relationship between temperature and rainfall. Thus forecasting temperature is almost tantamount to forecasting approximate rainfall. In the case of Bergen, Norway, in 22 consecutive years out of 23, June rainfalls were correctly predicted at least to the extent of being greater or less than in the preceding year, and in 13 years out of 25 the amount was predicted to within 25% (Table 2).

Mean February temperatures from Dalen i Telemark were used to predict mean June temperatures and rainfall from 1954 onwards for Kinlochewe, in Wester Ross. For temperature, the predictions were nearly as good as when February temperatures for Kinlochewe were used, but for rainfall they were usually better (Table 3). The particular case of 1974 is interesting. February was mild, and followed a quite exceptionally mild January. The predicted rainfall for June was 165 mm. In the event, the June rainfall was only 69.6 mm at Kinlochewe, but there was a very marked spell of wet weather, preceded and followed by dry conditions, commencing on May 18, the rainfall for this period was 164.6 mm, suggesting that the very mild and wet period in January–February was preconditioning a wet spell in May–June.

The meaning and physical explanation of this empirical prediction technique can only be guessed at. The second of Namias' factors—variations in the character of the land or sea surface—may be a more direct key to the relationships described than is the extraterrestrial factor. Can one determine, for instance, what was the particular state of which part of the Earth's surface in successive Februaries which injected a factor into the atmospheric circulation that was effective in inducing another particular condition the following June? It is not unreasonable to suppose that the surface conditions in February reflect the dominating influence which has characterised the whole winter.

Two significant factors which come to mind are (1) the generally accepted spread southwards of the Arctic high pressure

Table 2 Bergen (Frederiksberg), rainfall in June (mm)

	Forecast	Actual	Trend correct compared with previous year (C)	Forecast correct within 50 mm (C)
1950	160	203	—	C
1951	163	72	C	
1952	140	225	C	
1953	132	46	C	
1954	115	137	C	C
1955	95	57	C	C
1956	90	165	C	
1957	150	112	C	C
1958	100	26	C	
1959	155	183	C	C
1960	135	166	C	C
1961	180	223	C	C
1962	155	116	C	C
1963	80	68	C	C
1964	153	252	C	
1965	147	187	C	C
1966	105	106	C	C
1967	177	163	C	C
1968	125	132	C	C
1969	80	80	C	C
1970	100	78	C*	C
1971	175	69		
1972	153	247	C	
1973	157	130	C	C
1974	195	131	C	

* Actual precipitation almost the same as previous year

Table 3 Kinlochewe, June rainfall (mm)

	Forecast		Actual	Trend correct compared with previous year (C)		Forecast correct within 50 mm (C), within 25 mm (C*)	
	X	Y		X	Y	X	Y
1954	110	112	123	—	—	C*	C*
1955	125	102	82		C	C	C*
1956	120	75	75		C	C	C*
1957	140	145	82	C	C		
1958	135	82	60		C		C*
1959	170	125	201	C	C	C	
1960	110	170	190	C	C		C*
1961	175	147	137	C	C	C	C*
1962	165	170	190	C	C	C*	C*
1963	105	70	76	C	C	C	C*
1964	165	150	103	C	C		C
1965	145	165	164	C	C	C*	C*
1966	135	45	149	C	C	C*	
1967	175	147	124		C		C*
1968	110	120	120	C	C	C*	C*
1969	105	70	45	C	C		C*
1970	110	50	66	C	C	C	C*
1971	170	182	94	C	C		
1972	140	140	150	C	C	C*	C*
1973	140	182	177		C	C	C*
1974	165	180	70	C			

X—using Kinlochewe February mean temperature

Y—using Dalen i Telemark February mean temperature

area, and (2) the related decrease in westerly days in the zone to the south of this. It is significant that Trondheim, the northernmost station considered, showed the inverse February–June correlation earliest, and that it became recognisable further south and west as time went on. This implies increasing anticyclonic control of the relationship. Markedly anticyclonic and cold Februaries in Norway have almost invariably preceded warm Junes, and since well before the 1940s, but in addition it seems that milder, westerly Februaries now precede cool wet Junes more regularly than they used to.

In view of the very limited success of the methods at present used by the Meteorological Office in producing monthly weather outlooks³ I feel that studies of the type described here could be very useful ingredients in the development of long-range forecasting technique, especially when correlated with the *Grosswetter Lager* and other atmospheric circulation studies. I am pursuing this matter, with some success, in cases other than the February–June relationship.

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Molecular complexity of water vapour and the speed of sound

EVIDENCE has been given for a high binding energy of dimerisation in water vapour deduced from measurements of the speed of sound¹. The quoted value of 0.5 eV per molecule must be reconciled with values of about 0.2 eV per molecule which have been derived both from thermodynamic measurements² and from molecular orbital calculations³. We are interested in the behaviour of tropospheric water vapour, so we made observations at lower temperatures than the PVT and calorimetric observations which were relevant to steam technology. We

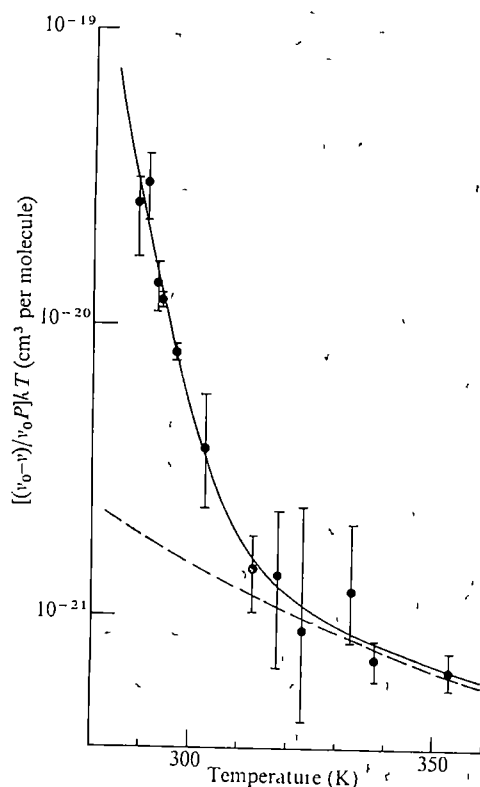


Fig. 1. $[(v_0 - v)/v_0 P] kT$ plotted against temperature. A two-dimer model fitted to the observations is represented by the solid line, for comparison, the dotted line is derived from published calorimetric measurements made in the temperature range 312–398 K. The points with error bars are derived from speed of sound measurements.

have now extended the temperature range of the speed of sound observations to cover the interval from 289 to 353 K.

Figure 1 shows values of the quantity $[(v_0 - v)/v_0 P] kT$ plotted against temperature T , where k is Boltzmann's constant. At each temperature the value of $(v_0 - v)/P$ was determined from the slope, and that of v_0 from the intercept of the straight line representing the observed speed of sound, v , against pressure, P .

Virial coefficients are used as the basis of the comparison of results, and are related to speed of sound by the expression⁴

$$\frac{(v_0 - v)}{v_0 P} kT = - \left[B + B'T(\gamma_0 - 1) + B''T^2 \frac{(\gamma_0 - 1)^2}{2\gamma_0} \right] \quad (1)$$

where B is the second virial coefficient, the primes denote differentiation with respect to temperature, and γ_0 is the ratio of specific heats for water at the limit of zero pressure. We take γ_0 to have the value 1.32. Only binary interactions have been considered, as within the limits of experimental error we have observed that changes in the speed of sound at a given temperature are linearly proportional to changes in pressure.

Published values of virial coefficients for water have been reviewed critically by Wexler and Greenspan⁵ and we have chosen for comparison those derived by Goff and Gratch⁶ from calorimetric measurements^{7,8} made in the temperature range

Table 1 Second virial coefficients (cm^3 per molecule)

	289 K	312 K	353 K
B_1 (Berthelot)	-8.3×10^{-22}	-7.0×10^{-22}	-5.4×10^{-22}
B_{2a}	-1.4×10^{-21}	-8.8×10^{-22}	-4.5×10^{-22}
B_{2b}	-2.8×10^{-22}	-5.1×10^{-24}	-1.4×10^{-26}
$B = B_1 + B_{2a} + B_{2b}$ (present work)	-2.5×10^{-21}	-1.6×10^{-21}	-9.9×10^{-22}
B (ref. 6)	-2.3×10^{-21}	-1.6×10^{-21}	-9.3×10^{-22}

from 312 to 398 K. The dotted curve in Fig. 1 is a plot of the function given by the right hand side of equation (1) using the empirical formula for the second virial coefficient, found by Goff and Gratch, to give the best fit to the calorimetric data. At temperatures where our observations overlap with the calorimetric data they lie close to the line but at lower temperatures they depart considerably from it.

The second virial coefficient, B , has been represented² as the sum of a dimerisation term, B_2 , and a term, B_1 , attributed to non-polar van der Waals' type attractions. Berthelot's formula allows B_1 to be calculated and for that formula we take the values of the critical temperature and pressure given by Rowlinson². For B_2 we use the expression

$$B_2 = - \frac{N_2}{N_1^2} = - \sigma \exp\left(-\frac{E}{kT}\right) \quad (2)$$

where N_1 is the concentration of monomers, N_2 of dimers, and $-E$ is the dimer binding energy. σ is a scale factor for dimer concentration, related to the partition functions of monomer and dimer, and is taken to have negligible variation with temperature. The Rowlinson formulation of the second virial coefficient with the values $-E = 0.17$ eV per molecule, and $\sigma = 1.4 \times 10^{-24}$ cm^3 per molecule gives a good fit to the calorimetric data.

To account for the speed of sound at low temperatures we have used a model for water vapour in which B_2 is itself the sum of two terms B_{2a} and B_{2b} , each applying to a species of dimer with a characteristic binding energy and scale factor. The full line in Fig. 1 represents a calculation using equation (1) and $B = B_1 + B_{2a} + B_{2b}$, with

$$\begin{aligned} -E_a &= 0.16 \pm 0.09 \text{ eV per molecule } (\sigma_a \sim 10^{-24} \text{ cm}^3 \text{ per molecule}) \\ -E_b &= 1.4 \pm 0.3 \text{ eV per molecule } (\sigma_b \sim 10^{-45} \text{ cm}^3 \text{ per molecule}) \end{aligned}$$

These were found by least squares analysis to give the best fit of the curve to our observations. Representative values of the second virial coefficient and its components are given in Table 1. In the temperature range we have studied, the values of B do not depart by more than the experimental uncertainty from values calculated from the empirical formula of Goff and Gratch⁶. At low temperatures, the significant difference between the two curves in Fig. 1 may be attributed instead to large values of $-B_{2b}$.

The binding energy of 0.5 eV per molecule given in the preliminary analysis in which only a single species was considered, is now explicable as a weighted average of the binding energies of two species of dimer. In our new analysis, values for the binding energy and the corresponding scale factor for one species are substantially in agreement with those of the familiar low binding energy dimer cited in the introduction. Comparing values of B_{2b} with those of B_{2a} we find that at 289 K the concentration of the high binding energy dimer is about 20% that of the low binding energy species. This indication of an appreciable concentration of dimers with a high binding energy is a provocative result which will need further investigation. We note that it applies to adiabatic conditions given by sound pulses which in our experiment had a duration of about 20 μs .

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Fusion ignition of microfission explosions

WINTERBERG¹ proposed that microexplosions could be caused in very small amounts of fissionable materials such as ²³³U, ²³⁵U, and ²³⁹Pu (see also refs 2 and 3). A small critical mass could be created by imploding the fissionable material until it was 200-300 times its normal density. This could be achieved using a high energy, properly shaped laser pulse (of about 1 ns duration). For a chain reaction to begin in the assembled critical mass, however, neutrons must be present to initiate the fission process. For a critical mass of uranium, of 0.34 g, and a hydrodynamic disassembly time of 10⁻⁹ s, as proposed by Winterberg¹, conventional neutron sources will not be adequate to initiate a chain reaction. Initiation of the fission process using neutrons produced from the fusion of a deuterium-tritium (D-T) mixture placed in the core of the fissionable material seems feasible and offers several distinct advantages over conventional neutron sources.

As far as conventional neutron sources are concerned, a beryllium- α source of sufficient strength to initiate a chain reaction within of the order of 1 ns would be too large to fit within the uranium sphere (it would have an initial radius of 0.16 cm). For example, Hanson⁴ gives strengths of the order of 2×10^7 neutrons s⁻¹ g⁻¹ of radium for Ra- α -n sources. To produce a neutron source strong enough to average 1 neutron ns⁻¹ (the hydrodynamic disassembly time) would take approximately 50 g of radium. Use of radon gas as an α source would require about 0.7 g of radon, which is still clearly unacceptable.

Other possible sources of neutrons, such as a Triga pulsed reactor, or a pulsed accelerator using a ⁹Be(d,n) reaction, would also be inadequate since, even if a high enough neutron flux could be achieved, the neutrons would strike the compressed pellet from the outside. That would be likely to initiate fission in the outer portions of the spherical mass before the desired degree of super-criticality had been reached, thus causing premature or non-uniform detonation, resulting in a fizzle yield.

As an alternative to these conventional neutron sources, a small amount of a D-T mixture could be embedded in the core of the sphere of fissionable material. Compression of the D-T core would induce thermonuclear fusion and produce 14 MeV neutrons according to the reaction



Neutrons from this reaction have been produced⁵ by the compression of a D-T pellet with a laser pulse. The rate of neutron production^{6,7} from this reaction is

$$dN/dt = N_D N_T (\bar{\sigma v})_{DT}$$

$$\text{where } N_D \simeq 2 \times 10^{26} \text{ cm}^{-3}$$

$$N_T \simeq 2 \times 10^{26} \text{ cm}^{-3} \quad (2)$$

$$(\bar{\sigma v})_{DT} \simeq 10^{-21} \text{ cm}^3 \text{ s}^{-1}$$

are the deuterium number density, tritium number density and Maxwell-Boltzmann average of the product of

the cross section and velocity, respectively. A D-T mixture occupying 10⁻⁶ of the volume of the sphere of fissionable material gives 10¹² neutrons in 10⁻¹¹ s (the approximate neutron doubling time in the fission reaction). So the D-T fusion reaction will produce copious quantities of neutrons in the necessary time interval, and will initiate the fission reaction.

There are many advantages to this technique of initiating fusion. First, having the D-T mixture in the core of the spherical critical mass makes the initial neutron density highest at the centre of the sphere. It decreases radially outward, leading to an efficient chain reaction which develops from the middle towards the edges. Second, at the centre of the sphere there would be a pressure spike from the implosion of the sphere to critical mass, this higher pressure (and density) would make the production of neutrons by D-T fusion more efficient. Finally, since the core would be the last part of the sphere to be compressed, no neutrons would be produced until a critical (or super critical) mass was achieved. That would lead to high efficiency and would avoid the problems of a fizzle yield.

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Dissolution of powdered quartz

POWDERED quartz exhibits an initially high solubility rate but opinions are divided as to the cause. Clelland¹ argued in favour of the 'Beilby layer'—a skin of vitreous material produced during grinding, whereas Talbot² stated that the numerous small particles which were always found adhering to the larger particles of quartz offered a plausible explanation.

The fraction of quartz detectable by differential thermal analysis (DTA) becomes less³ during grinding, but the fraction can be restored to 100% by etching with hydrofluoric acid. This has been attributed to the dissolution of amorphous layers from the surfaces of the particles, leaving behind crystalline quartz. We show here, however, that when the fraction of quartz detectable by DTA is increased as a result of etching in hydrofluoric acid, the material which dissolves is crystalline and not amorphous.

A quartz sand from Chatteris, Cambridgeshire, produced a characteristic DTA inversion peak (Fig 1a). The quartz sand was milled for 400 h in a vibration mill with a steel body containing steel balls, and iron contaminant was removed from the resultant powder using hydrochloric acid. The purified material produced a curve with a greatly reduced DTA inversion peak (Fig 1b). The material was then subjected to two different treatments.

The first involved etching in dilute hydrofluoric acid which resulted in the dissolution of 30% by weight of the material. The residue was collected by centrifugation and produced a DTA curve with a larger inversion peak (Fig 1c).

The second treatment involved separating the powder into a coarse and a fine fraction by dispersing the quartz in dilute hydrochloric acid (quartz is less soluble in hydrochloric acid than in water and is easier to disperse) and centrifuging the suspension to obtain a precipitate of the

coarser material, leaving the finer material in suspension. The process of dispersal and centrifugation was repeated until 30% by weight of finer material had been separated out. The coarse fraction thus obtained was subjected to DTA, and produced a curve with an inversion peak that had increased in size (Fig 1d) compared with the original material (Fig 1b) but, which was, perhaps, not so large as that produced by the etched quartz (Fig 1c).

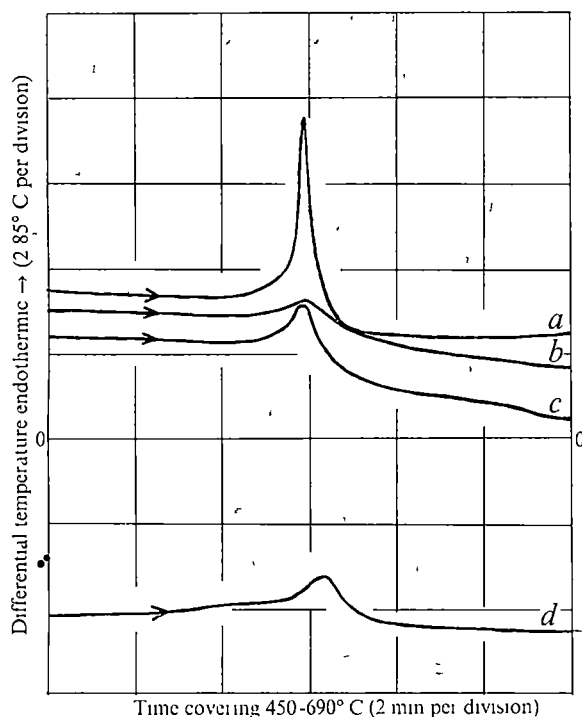


Fig 1 Differential thermal analysis curves of an 0.285 g sample of powdered quartz heated at $20^{\circ}\text{C min}^{-1}$. a, original quartz sand, b, after grinding for a duration of 400 h, c, ground material from b after etching, d, ground material from b after the fines had been removed by sedimentation.

The fine material was subjected to two tests. First, it was suspended in hydrofluoric acid of the same strength as that used during etching. It dissolved completely within the time that had been allowed for the etching treatment. Second, a small quantity of the finest material obtained (less than $0.1\text{ }\mu\text{m}$ Stoke's diameter) was tested by X-ray diffraction (Fig 2a) and found to consist substantially of crystalline quartz. A reference picture (Fig 2b) for quartz milled for 3 h is also given. The fine material, shows a small amount of line broadening, but displays peak intensities almost equal to that of the reference sample.

Since the fine material dissolved completely within the time previously allotted for etching, it seems that similar fine material must have been removed by the hydrofluoric acid, leaving behind the larger particles of quartz. Furthermore, these fine particles must consist substantially of crystalline quartz (Fig 2a and b) and are therefore not amorphous. Thus, since the removal of fine particles by sedimentation resulted in a larger DTA peak, the increase in size of DTA peak as a result of etching the powder in hydrofluoric acid must to some extent be caused by the removal of fine particles which dissolve during the etching treatment.

Does the inversion peak produced by the etched material, which is larger than that produced by the coarse material obtained by sedimentation, leave a margin of uncertainty which could be explicable in terms of the dissolution of an amorphous layer? There are three answers to this question.

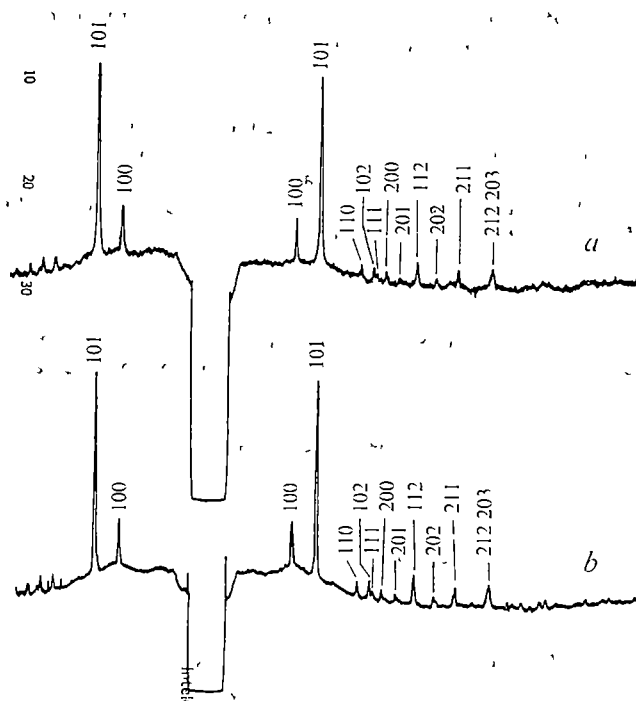
First, the processes of removing finer material by sedimentation and by etching cannot be assumed to be exactly equivalent. The powder is very difficult to disperse and sedimentation allows some very fine particles to remain attached to the larger ones. Furthermore, there is evidence (G S M M and H E R, unpublished) that during the grinding of quartz, 'internal subdivision' occurs resulting in the formation of grains within each particle. Such a system would be very difficult to disperse but finer grains and particles would not escape more rapid dissolution in hydrofluoric acid.

Second, the grinding of the quartz could produce residual strain within the particles. The inversion temperature of quartz is a function of pressure, and residual strain could cause the DTA inversion peak to be less pronounced if the inversion is spread over a range of temperature as a result of residual strain. When etched in hydrofluoric acid, the removal of the material under a state of strain, presumably more soluble in this condition, could produce a release of strain from the remaining quartz thus causing a more pronounced DTA inversion peak.

Third, we have found that, according to the results of density measurements, there is no evidence that amorphous material is produced, even when the quartz had been dry ground for several hundred hours, until crystalline quartz could not be detected by DTA.

The density of the powder, after corrections for absorbed atmospheric moisture and impurities, remained at 2.65 g ml^{-1} during grinding, and X-ray diffraction confirmed that the powder had a crystalline structure corresponding to that of quartz. Thus, although the quartz remained in a crystalline condition during grinding, it was apparent that the inversion characteristics had become so modified that the DTA inversion peak at 573°C , normally characteristic of quartz, had disappeared. So, it seems that during etching, this finely divided quartz which we have referred to as

Fig 2 Photodensitometer traverses of X-ray film a, material with a particle size less than $0.1\text{ }\mu\text{m}$ extracted from quartz ground for 400 h, b, a reference curve of quartz milled for 3 h. Cu $K\alpha$ radiation, Ni filter.



of females might be genetically too inflexible. The extreme in maximisation of brood space would be for all members of the population to produce eggs, some or all of them producing sperm also. (Carlquist¹⁰ reasons similarly regarding gynodioecious plants.)

This last possibility, simultaneous hermaphroditism, occurs in the only other species of externally-brooding actinian to have been studied¹¹, called *E. prolifera* at that time, this species is now identified as *E. japonica* (T. Uchida, personal communication). Selection against sperm production beyond what is necessary to fertilise sufficient ova to fill the brood space by restricting it to certain members of the population is a possible evolutionary pathway from simultaneous to gynodioecious hermaphroditism. The larger anemones might have been favoured as sperm-producers since they had proved best adapted by virtue of their longevity, and because proportionately less of the energy and material budgeted for gametes could be used by them for sperm than by smaller animals.

Developmental gynodioecy in self fertile species would favour reproduction by only the fittest colonists of new habitats, maximising the chances of survival of succeeding generations. If a hermaphrodite were the founder, it could begin to establish a population immediately. If the animal had not yet attained hermaphroditism, it could begin to reproduce successfully only after having proved its fitness for the new circumstances by surviving in them. Even assuming *E. prolifera* can self, however, it must rarely survive being swept into a new site, so this advantage probably had little to do with the evolution of gynodioecy in this species.

Developmental gynodioecy is an adaptable breeding system. Theoretically, the genetic diversity of gametes is as great as in simultaneous hermaphrodites because virtually all individuals eventually produce sperm as well as ova. Excessive sperm production can be prevented by evolutionary adjustment of the time of onset and extent of testicular development to optimise the sperm:egg ratio of the population. At any one time, the same ratio can be attained as in dioecious species but with greater genetic diversity of sperm because more hermaphrodites are required to produce as many sperm as can be produced by a given number of purely male individuals of the same size.

The genetically-fixed gynodioecy of plants apparently evolved as a mechanism for enhancing outbreeding among normally self fertile hermaphrodites. The developmental gynodioecy of *Epiactis prolifera* probably evolved as a means of increasing the number of brooders (egg-producers) in a population with limited fecundity to a maximum, and possibly also of enhancing inbreeding among normally cross fertile individuals.

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Evidence for a Y chromosomal contribution to an aggressive phenotype in inbred mice

IN man the XYY chromosome complement has been reported by some authors to be associated with abnormal tallness, mental deficiency and sociopathic, aggressive behaviour¹⁻⁴. The interpretation of this literature is controversial. Furthermore, any genetic variation(s) of the Y chromosome which affect(s) aggressive behaviour among chromosomally normal males could mask the correlation between a supernumerary Y and this behaviour⁵. If such variation exists, then in any population the correlation between the XYY karyotype and aggression would depend on the distribution of Y chromosomes having varying degrees of predisposition towards aggressive behaviour. Our investigations on the developmental genetics of fighting behaviour in mice support the hypothesis that there may be heritable variations of the Y chromosome which are associated to varying degrees with some types of aggression, and that this could account both for the lack of unusual aggressive behaviour in many individuals with an extra Y and for its occurrence in other XYY males.

In an initial study, one of us⁶ showed that male DBA/1/Bg mice were more aggressive than C57BL/10/Bg males and that in their reciprocal hybrids, those F₁ males sired by DBA/1/Bg were more aggressive than those sired by C57BL/10/Bg. These findings, suggested to us that the Y chromosome of the DBA/1/Bg makes an incremental contribution to intermale aggressiveness. We report here the original data⁶ and a replication entailing a larger sample.

Intraspecific aggression was measured in encounters between pairs of male mice isolated from the time of weaning at 29 d of age until testing at 50 d of age when they were sexually mature. Pasteurised food and water were available *ad libitum*. Water was acidified (pH 2.5) and chlorinated (12-18 p.p.m.). Lighting was on a 12 h light: 12 h dark diurnal cycle with lights on at 0600. The mice were tested in a laboratory cage (16 × 26.5 × 11.5 cm) covered by a 6.4 mm wire mesh. The cage was divided by an opaque partition. Subject animals were placed on either side of the partition which was removed after a 5-min adaptation period. Trials were limited to 10 min during which behavioural records were kept for each 20 s interval. Each pair was tested daily for three consecutive days. Agonistic acts recorded consisted of tail rattling, wrestling, flank biting, chasing or full attack⁷⁻⁹. Any aggressive act by one mouse during a 20-s interval was scored as '1'; if both mice reacted aggressively during the same period, it was scored '2'. Aggression scores were obtained by summing the pair scores over the three trials and averaging these scores for each genotype.

Table 1 Reciprocal effects on aggressive behaviour

		Initial*		C57BL/10 hersFat
		DBA/A Fathers		
DBA/1 Mothers		34.3 ± 5.1† (10)‡		21.5 ± 5.5 (10)
C57BL/10 Mothers		34.0 ± 3.0 (10)		5.0 ± 2.1 (10)
DBA/1	B10D1F1		D1B10F1	C57BL/10§
		34.3	34.0¶	21.5
		Replication		
		DBA/1 Fathers		C57BL/10 Fathers
DBA/1 Mothers		35.4 ± 4.6 (20)		10.7 ± 2.2 (42)
C57BL/10 Mothers		19.8 ± 3.0 (43)		7.3 ± 2.3 (22)
DBA/1	B10D1F1		D1B10F1	C57BL/10
		35.4	19.8	10.7
				7.3

* The initial study was carried out at the University of Chicago between 1967 and 1968 and the replication was done at the University of Connecticut between 1972 and 1973.

† $\bar{X} \pm \text{s.e.m.}$; maximum possible aggression score was 180 for both studies.

‡ Number of male pairs tested.

§ Neuman-Keuls analysis of aggression score means; $P < 0.05$ (after Winer¹⁰).

¶ Underlined means are statistically equivalent.

Table 2 Matherian scaling of aggression scores

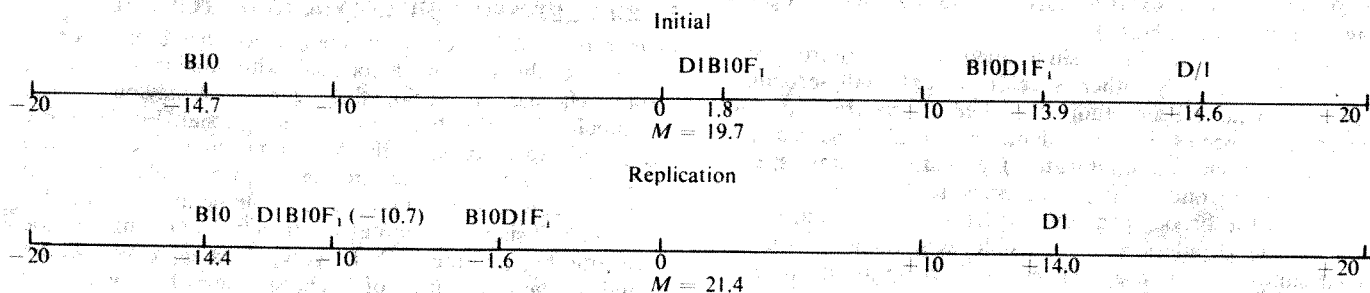


Table 1 indicates the aggression score obtained in the two experiments. The scores for each parental strain and thus for the mid-parental values are essentially unchanged. In the initial study, a marked but non-significant difference ($P < 0.10$) was noted between the two reciprocal F_1 mice. This difference attained significance in the replication ($P < 0.05$; Student's t test). Analysis of variance for all four genotypes indicates a highly significant F statistic for both samples ($P < 0.001$). A Neuman-Keuls analysis designates the significant differences between genotypes (Table 1). In the initial study, the score of the B10D1F₁ is similar to that of the DBA/1 strain; whereas in the replication, it is closer to that of the mid-parent. In the same study, the score of the D1B10F₁ is similar to that of the mid-parental value; whereas in the replication, it is closer to that of the C57BL/10/Bg strain. These relationships can be seen more clearly on a Matherian scale (Table 2) where the aggression score of each genotype is represented as a deviation from the mid-parental value¹⁰. In both samples, the reciprocal differences are in the same direction, and their magnitude, measured as deviations from the mid-parental values, are essentially the same.

A possible autosomal contribution to aggressive behaviour was detected in the original study⁶, in a study by Eleftheriou *et al.*¹¹; but not in the present one. The factors accounting for this discrepancy are possibly that the animals used in the replication, though of the same substrain (Bg), were at least 20 generations removed from those used in the initial study. They were also maintained under specific pathogen-free conditions, while the original animals were housed in a conventional colony. That the Y chromosomal contribution could be replicated in the hands of a second investigator under somewhat different environmental and testing conditions suggests that the latter effect is more robust.

Since the DBA/1/Bg mice are more aggressive than the C57BL/10/Bg mice, and since the magnitude of the F_1 scores is associated with that of the male parent, it is a reasonable hypothesis that the reciprocal differences in aggressiveness are the result of the reciprocal differences in the origin of the Y chromosome, as previously suggested¹². Because the more aggressive hybrids developed in the uterine and postnatal environment of the less aggressive C57BL/10/Bg strain, these maternal conditions seem to make no major contribution to the aggressive behaviour of these strains and hybrids.

Although there seems to be an incremental effect of the Y chromosome derived from DBA/1/Bg mice on aggressive behaviour, our studies indicate that the Y chromosome of the aggressive DBA/2/Bg strain does not potentiate intermale fighting when analysed in similar crosses. Also, in reciprocal crosses between CFW and A/J mice, Southwick¹³ reported that F_1 mice sired by the more aggressive strain (CFW) were not more aggressive than their reciprocal F_1 generation. Thus, the variation in intermale fighting in mice is very likely under the control of a variety of genetic factors, including the Y chromosome.

These results add to the growing evidence¹⁴⁻¹⁷ which suggests

that the Y chromosome, in at least one mammalian species, is not inert and that extrapolating from mouse to man, there may be active genes on the Y for traits other than hairy ears, foot ulcers and radio-ulnar synostosis¹⁸. Our hypothesis thus places constraints on inferring behavioural potential from the XYY karyotype of man or other mammals.

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Response of flying mole crickets to three parameters of synthetic songs broadcast outdoors

LARGE numbers of flying crickets can be attracted to outdoor loudspeakers broadcasting male calling songs¹. Consequently we have been able to investigate the features of calling songs that are important for attraction. Previous investigators have used laboratory-maintained crickets in acoustical environments significantly different from outdoors²⁻⁷. Furthermore, they have studied thoroughly no more than one or two of the important signal parameters. We have studied outdoors the responses of free-flying mole crickets, *Scapteriscus acletus*, to synthetic call-

ing songs systematically varied in these three parameters: carrier frequency, pulse rate and intensity. Mole crickets came in greatest numbers when we broadcast, at unnaturally high intensities, carrier frequencies and pulse rates like those of the natural song.

All experiments were conducted near Gainesville, Florida, during 1972 and 1973. During the tests soil temperatures were $24 \pm 4^\circ \text{C}$, air temperatures, $25 \pm 5^\circ \text{C}$, and relative humidity, $70 \pm 20\%$ (ref. 8). We used three independent broadcasting systems, each with its own cricket-trapping funnel. A fourth funnel, without a speaker, was the control. Two test sounds and a standard sound were broadcast simultaneously. Test sounds were synthetic songs (artificial sounds synthesised and tape-recorded in the laboratory) varied in intensity, carrier frequency and pulse rate (that is, rate at which the carrier was turned on and off). Equal durations of pulses and pulse intervals were maintained. For frequency experiments (1972), the pulse rate and intensity were held constant at 60 pulses s^{-1} and 100 dB sound pressure level (SPL) (at 15 cm; reference SPL, $2 \times 10^{-5} \text{ N m}^{-2}$). Similarly, in pulse rate experiments (1973), the frequency and intensity were held constant at 2.7 kHz and 100 dB. In intensity experiments (1973), frequency and pulse rate were constant at 2.7 kHz and 55 pulses s^{-1} . The standard sound was 2.7 kHz and 100 dB. It was changed from 60 pulses s^{-1} in 1972 to 55 pulses s^{-1} in 1973 to approximate more closely the natural song (that is, the actual calling song of males in the field) at 25°C . The number of mole crickets trapped at each test sound was expressed as a percentage of the number caught at the standard sound. Trials with less than 10 crickets in the standard were discarded. Each test sound was used on at least two nights. We have described the trapping equipment and the broadcasting techniques before¹.

The control funnel generally caught no mole crickets and never had more than 1.5% of the number of adults in the other three funnels. In frequency and pulse rate trials the number of trapped mole crickets varied fivefold about a mode (Fig. 1). For every 6-dB increase in sound level up to 106 dB, our catch approximately doubled (Fig. 2). In addition to comparing seven pulse rates, we investigated whether any amplitude modulation at all was necessary for phonotaxis of *S. acletus*¹⁰. We made four trials comparing a continuous tone (no amplitude modulation) of 2.7 kHz with the 1973 standard sound (tone turned on and off 55 times per second). Because 270 *S. acletus* were captured at the standard sound, 27 at the continuous tone, and none at the control, amplitude modulation must be an important, but not essential, feature.

Several features of male calling songs are known or suspected to cause species-specific responses in crickets²⁻⁷. For mole crickets, Bennet-Clark¹¹ suggested that carrier frequency might

Fig. 1 The response of flying *S. acletus* to synthetic sounds that were systematically varied in frequency (a) and pulse rate (b). The relative response is a percentage of those captured at the standard sound (circle and cross) (see text). Vertical bar is 2 s.e. on either side of the mean. Narrow vertical line is range. In 16 frequency trials, 416 individuals were trapped; in 24 pulse rate trials, 843 were trapped.

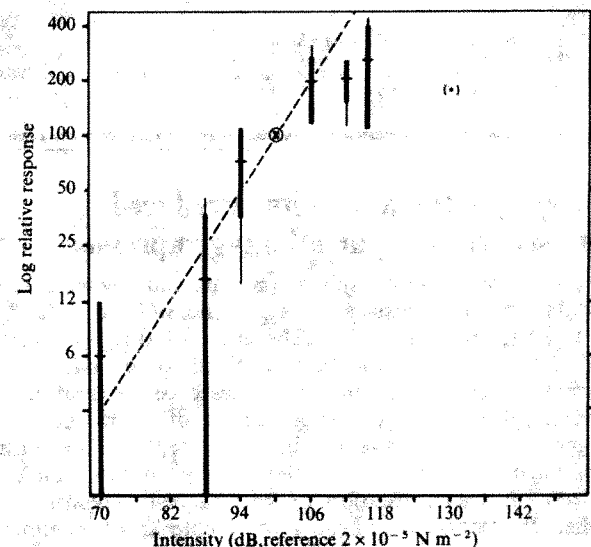
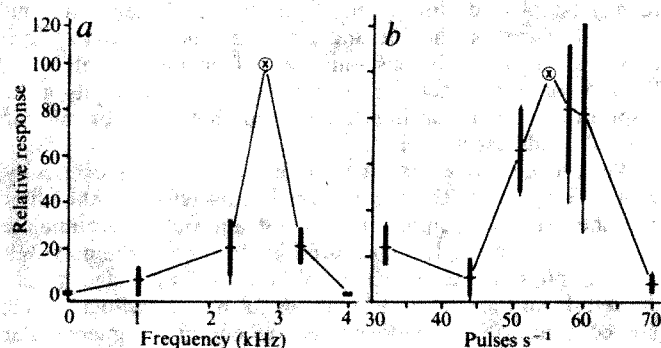


Fig. 2 The response of flying *S. acletus* to various intensities. Relative response is a percentage of those captured at the standard level (circle and cross, 100 dB). (●) Represents result of a single trial with a different speaker. The dotted line represents a doubling of the number for each 6 dB increase in sound pressure level. Vertical bar is 2 s.e. on either side of mean. Narrow vertical line is range. In 33 intensity trials, 1,606 individuals were captured.

be important to flying females. Our data (Figs 1 and 2) demonstrate that both carrier frequency and pulse are important to species-specific phonotaxis in *S. acletus*. This insect (Orthoptera, Gryllotalpidae) should be able to separate its own song from songs of most other crickets by pulse rate (Fig. 1); however, there are crickets occurring in the same habitat with pulse rates overlapping those of *S. acletus*. *S. acletus* could distinguish the songs of its own males from the songs of these other crickets by carrier frequency, since carrier frequency of these songs is higher than in *S. acletus*¹.

Doubling of catch for each 6-dB increase in sound level agrees with the following model. Sound waves radiate in all directions from loudspeakers, and sound pressure is generally halved (that is, drops 6 dB) for each doubling of distance from a sound source. Whatever the shape of the sound field, its diameter would double for each 6-dB increase in sound level. To orientate to a calling song, the flying adults must first encounter an appropriate sound above a threshold pressure. If mole crickets fly in a single plane and maintain straight courses, the catch should double for each doubling of diameter (not the area) of the sound field. The failure of doubling to continue with each increase of 6 dB beyond 106 dB could result from the crickets being repelled by such high intensities or from an inability to orientate owing to saturation of their auditory organs.

The synthetic songs that trapped maximal numbers of mole crickets had pulse rates and frequencies like those of the natural song but the catch was increased 30-fold at intensities 38 dB or more above natural intensities, which average 68 dB⁹. Such attraction may prove useful in control of these agricultural and turf pests.

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Ripening of tomato fruits at reduced atmospheric and partial oxygen pressures

STORAGE at reduced atmospheric pressure and low temperature is used to retard the post-harvest senescence of fruits, vegetables, cut flowers, and cuttings¹⁻³. The effect of this low pressure storage (LPS) has mainly been ascribed to reduction of the internal concentration of the senescence-accelerating gas ethylene⁴, although occasionally effects of a lowered partial oxygen tension (P_{O_2}) have also been reported⁵⁻⁷. To determine the contribution of the reduction in P_{O_2} to the total effect of LPS on the retardation of post-harvest senescence, we studied the course of ripening of tomato fruits at different atmospheric and partial oxygen pressures, with and without absorbing the ethylene produced. We report that both the onset and the rate of the ripening process are determined by the oxygen pressure, irrespective of the atmospheric pressure and the absorption of endogenously evolved ethylene.

Tomato fruits of the Jupiter cultivar were grown under greenhouse conditions and carefully selected from the same position at comparable clusters. Samples of 15 mature green fruits were placed in 21-l desiccators over dry lime to absorb the carbon dioxide produced. Part of the desiccators also contained a 3% potassium permanganate solution in 1 N sulphuric acid, to absorb the ethylene evolved. The ethylene concentration in the desiccators was measured daily using a gas chromatograph. After gas sampling the desiccators were flushed to refresh the atmosphere completely. Gas mixtures were applied from cylinders and the atmospheric pressure adjusted by suction. When the fruits were in their climacteric stage, respiratory oxygen consumption never reduced P_{O_2} below 0.18 and 0.037 at initial P_{O_2} values of 0.21 and 0.04, respectively. The respiration rate at $P_{O_2} = 0.1$ was still equal to that at $P_{O_2} = 0.2$, but at a pressure of 4% it must be considerably reduced. The onset and rate of ripening were followed daily using visual colour determination, and at the table-ripe stage the softness of the fruits was also measured⁸. The weight loss never exceeded 2%. Table 1 summarises the results of an experiment at 19° C, under diffuse light from fluorescent tubes.

The normal period taken to ripen, 7 days, is delayed by pure oxygen at $P_{O_2} = 1.0$; also the lycopene synthesis is disturbed, the ripe soft fruits becoming intensely orange but not red. At about $P_{O_2} = 0.2$, however, the presence of other atmospheric components fails to affect ripening, the fruits ripening in 7 days both at normal and at reduced atmospheric pressure, whether or not the ethylene is absorbed. Even at 19 cm Hg and in the presence of permanganate solution, the ethylene level does not limit the rate of ripening, whereas at the reduced oxygen level of 5%, ripening is considerably retarded, even without absorption of ethylene.

Table 2 shows an experiment at two temperatures, 19° C and 13° C. Again, at the normal P_{O_2} of 0.2 the usual ripening periods of 9 and 17 days occur at 19° C and 13° C, respectively,

Table 1 Rate of ripening of Jupiter tomato fruits, from the mature green to the table-ripe stage, at different atmospheric conditions at 19° C

Pressure (cm Hg)	Atmosphere	P_{O_2}	KMnO ₄	Days to ripeness
76	O ₂	1.0	+	13
			—	13
76	Air	0.21	+	7
			—	7
19	O ₂	0.25	+	7
			—	7
19	Air	0.05	+	27
			—	27

Table 2 Rate of ripening of Jupiter tomato fruits, from the mature green to the table-ripe stage, at different atmospheric conditions at two temperatures

cmHg	Atmosphere	P_{O_2}	KMnO ₄	Days to ripeness 19° C	13° C
76	Air	0.2	+	9	17
			—	9	17
15	O ₂	0.2	+	9	16
			—	9	17
15	Air	0.04	+	24	28
			—	24	28
76	N ₂ +O ₂	0.04	+	29	32
			—	29	32

regardless of the total atmospheric pressure and the absorption of ethylene. In the presence of permanganate solution, the level of ethylene never exceeded $2 \mu\text{l l}^{-1}$ and was usually much less than $1 \mu\text{l l}^{-1}$. Without ethylene absorption, the ethylene level at 20% oxygen surpassed the threshold value (ref. 9 $5 \mu\text{l l}^{-1}$) for ripening of tomato fruit within 2 days; at the reduced oxygen level of 4%, on the other hand, the ethylene level always remained below $4 \mu\text{l l}^{-1}$, mostly below $2 \mu\text{l l}^{-1}$, and the ripening was considerably retarded in all cases. The lack of oxygen is the overriding factor at $P_{O_2} = 0.04$, determining the rate of ripening. At normal atmospheric pressure the ripening was even more delayed than at reduced pressure.

That not only the rate, but also the onset, of ripening is delayed at a low partial oxygen pressure is shown in an experiment with earlier harvested fruits (Table 3). In air at normal pressure, the fruits started ripening after 4 days and reached the pink stage in 8 days. The reduced partial oxygen pressure of 4% postponed the onset of ripening considerably, again at an atmospheric pressure of 76 cm more than at 15 cm. The conclusion that oxygen is the limiting factor at this concentration, rather than ethylene, is supported by the fact that the presence of the permanganate solution is irrelevant.

Table 3 Delay of onset of ripening, to the first sign of colour breaking (stage 2), at different atmospheric conditions at 19° C

cm Hg	Atmosphere	P_{O_2}	KMnO ₄	Days to stage 2
76	Air	0.2	+	4
			—	4
76	N ₂ +O ₂	0.04	+	16
			—	16
15	Air	0.04	+	10
			—	13

We have therefore confirmed the usefulness of LPS in delaying post-harvest senescence. Contrary to earlier indications¹, however, we have clearly demonstrated that, at least for the commodity and conditions used, the effect of LPS is not primarily caused by a reduced ethylene concentration, but rather by the low partial oxygen pressure. In this respect LPS acts in a similar way to the well established method of controlled atmosphere storage, in which senescence is retarded by storage in an atmosphere reduced in oxygen and enriched in carbon dioxide by the respiratory activity of the commodity¹⁰⁻¹². Among the advantages of LPS are the more readily established atmospheric conditions and the continuous removal of carbon dioxide and ethylene. The reduced oxygen pressure may affect the onset and rate of ripening by inhibiting the biosynthesis or activity of ethylene^{12,13}, or by limiting the respiratory activity or interfering with the oxidation of, for example, indoleacetic acid¹⁴.

We also conclude that the concentration of endogenously evolved ethylene in the atmosphere has no effect on the onset and the rate of ripening, if it is not allowed to accumulate excessively. The ethylene produced in ripening tissue may well exert its physiological effect mainly during its passage, within the cell, from the site of biosynthesis to the intercellular space. The concentration of endogenous ethylene in the intercellular space is thus an indicator rather than an effector of ripening

and its reduction by low atmospheric pressure or absorption is not effective in regulating senescence.

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Blue-green algae associated with ascidians of the Great Barrier Reef

In the rich biota of reef communities, one of the best known symbiotic relationships is that of dinoflagellates known as "zooxanthellae" with corals and giant clams^{1–3}. In contrast, the presence of algae in ascidians (sea squirts: Phylum Chordata, Subphylum Tunicata), although known for many years, has been studied very little. It is known that the association is confined to tropical ascidians in the family Didemnidae, but even the phylum to which the algae belong, or indeed whether the green cells in question are algae at all, has remained uncertain. During a recent expedition of the RV Alpha Helix to the Great Barrier Reef, we encountered several species of colonial ascidians containing large numbers of bright green, spherical cells. We have established by optical and electron microscopy that these green cells are blue-green algae; their association with primitive chordates represents a considerable extension of the known host range of these prokaryotes.

Our collections were made between March 25 and May 13, 1973, on reefs surrounding Lizard, Nymph and Turtle Islands off the north-east coast of Queensland (14°40'S, 145°30'E). Of the species of ascidians we collected five belong to the Didemnidae (Patricia Kott Mather, personal communication). We found that three of these [*Didemnum ternatanum* (Gottsch.), *Diplosoma virens* (Hartmeyer), and *Lissoclinum molle* (Herdman)] contained large numbers of algae. *Didemnum ternatanum* is a brown-coated, usually urn-shaped species 1–3 cm in height, while *Diplosoma virens* and *Lissoclinum molle* are blue and green encrusting forms, respectively. In all three ascidians the seawater strained of its plankton by the zooids collects in the labyrinthine central cloacal cavity of the colony, from which it is expelled through common excurrent pores. The algae are located extracellularly within cloacal pockets and folds around the zooids, or appressed to strands of the cellulose-like matrix or test that span the cloacal cavity (Fig. 1).

Earlier observations on the algae of didemnid ascidians had identified them as either zooxanthellae^{4,5} or zoochlorellae⁶, the latter category including various green algae associated with animals⁷. We concluded from light microscopy, however, that the algae in our ascidians are blue-greens, since they lack discernible nuclei and form new cells by wall ingrowth and constriction. These algae are bright green, their absorption spectra indicating the presence of chlorophyll *a* and little or no phycocyanin and phycoerythrins (T. Akazawa, personal communication). All three algae are unicellular and spherical, but in other respects differ sufficiently to be readily distin-

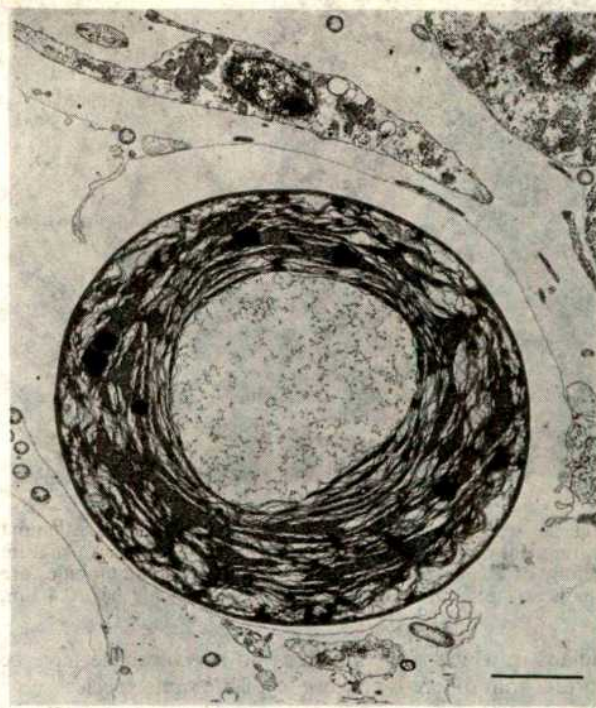


Fig. 1 Thin section through a coccoid blue-green alga lying in the cloacal cavity of the colonial tunicate *Diplosoma virens*. This electron micrograph illustrates the concentric lamellae, polyhedral inclusions and clear central area of the alga. The alga resides in a pocket formed by the test of the colony. Cells of a zooid can be seen at top right. Bacteria are embedded among the cellulosic fibrils of the test at lower left and elsewhere. Scale bar, 2 μ m.

guishable from one another. The algae of *Diplosoma virens* and *Lissoclinum molle* usually contain a clear central region not seen in the algae of *Didemnum ternatanum*. Also, the algae of *D. virens* range in diameter from 7–15 μ m whereas the algae from *D. ternatanum* and *L. molle* average about 20 μ m.

The three algae have a basically similar ultrastructural organisation but differ in detail. The cells lack mitochondria and other eukaryotic organelles, and their fine structural features are characteristic of blue-green algae (Figs 2 and 3a). Since the fine structural features are quite constant between algae from different collections of the same species of ascidian, a single micrograph is usually sufficient to enable identification of the alga in terms of its ascidian host (compare Figs 2 and 3b). On the basis of classical taxonomic features determined with the optical microscope, the algae from formalin-preserved colonies of all three ascidians have been identified as the single blue-green species, *Anacystis aeruginosa* (Zanard.) Drouet & Daily (Francis Drouet, personal communication). But, the consistent fine structural differences suggest that the three algae may represent different species, each associated with its particular species of ascidian.

There are reports of the presence of algae-like cells in the cloacal cavities of colonial ascidians. Herdman⁸ in 1906 mentioned their presence in the cloacae of *Diplosoma virens* and *D. viride* (= *D. virens*) from Ceylon. On the Yonge expedition to the Great Barrier Reef in 1928–29, Hastings⁴ observed brownish algal inhabitants in the cloacal cavities of three didemnid ascidians, including *D. virens* and *Lissoclinum molle*. The algae were identified as zooxanthellae by Yonge⁴ whose identification was corroborated by Smith⁵ from preserved specimens. Smith also pointed out, however, that in a collection of *D. virens* from Ceylon the algae, being green, were possibly zoochlorellae. More recently, Tokioka⁶ examined ascidians from the Pacific Ocean in the United States National Museum and found that seven didemnids harboured algae, all of which were identified as zoochlorellae. Thus there are several didemnid

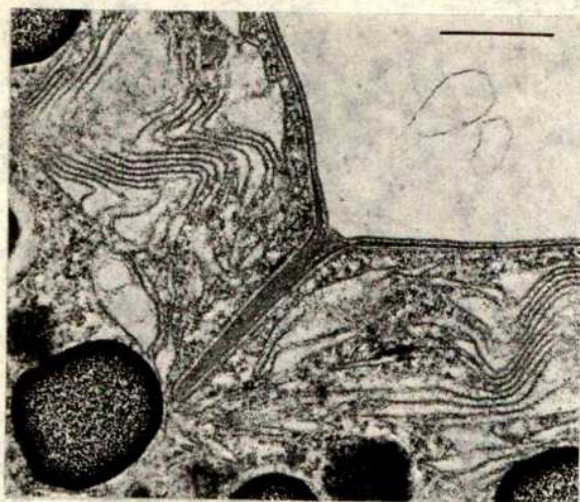


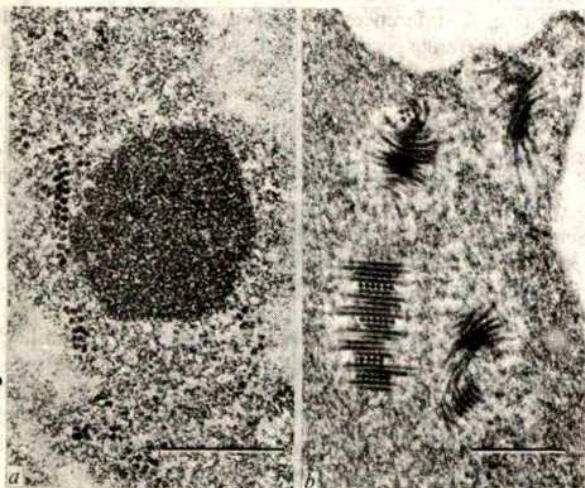
Fig. 2 Ingrowth of the wall bounded by the plasmalemma during cell division by constriction in the alga from a mature colony of *Didemnum ternatanum*. The osmiophilic globules are characteristic of the algae in this ascidian. Scale bar, 0.4 μ m.

ascidians in which zooxanthellae and zoochlorellae have been reported from different collections of the same species.

We conclude from our observations that some or all of the zoochlorellae reported in ascidians by past workers may have been blue-green algae. In any event it seems clear that in several cases a particular ascidian species is capable of harbouring either of two quite different algae. Thus *Diplosoma virens* and *Lissoclinum molle* collected at Low Isles on the Yonge Expedition contained zooxanthellae, while the same species collected by us approximately 115 miles north of Low Isles (and 45 years later!) contained blue-green algae. There is no evidence that mixtures of the two kinds of algae occur. In examining thousands of algae and the tissues of many zooids in our didemnid ascidians, we have not found a single zooxanthella, although zooxanthellae exist in enormous numbers in the nearby corals and clams.

Although earlier reports have established the presence of either zooxanthellae or zoochlorellae in different collections of *Trididemnum cyclops*⁴⁻⁶, the presence of algae may not be necessary for the existence of this ascidian at least, as we found flourishing colonies of this species growing without algae on the underside of a coral rock. Whether the ascidians utilise photo-

Fig. 3 a, Particles suggestive of ribosomes helically disposed in a polysome lie adjacent to a polyhedral inclusion in an alga from *Lissoclinum molle*. All of these structures are common to the algae of the three ascidians. Scale bar, 0.3 μ m. b, Characteristic paracrystalline arrays of electron opaque structures arranged as rods and arcs in an alga from *Lissoclinum molle*. Scale bar, 0.3 μ m.



synthetic products of the algae, as do clams and corals, has not yet been established. Tests for nitrogen fixation by the algae were negative (R. H. Burris, personal communication).

Previously, blue-green algae in the marine environment have been identified as symbionts only of certain diatoms¹⁻³ and a very few marine invertebrates, among which are certain sponges⁹⁻¹². The association we observed is therefore of special interest since it greatly extends the host range of blue-green algae to include primitive members of the Chordata, and thus represents an association with marine animals considerably higher in the evolutionary scale than hitherto recognised.

Our formalin-preserved ascidians have been deposited in the Queensland Museum; we thank Dr Patricia Kott Mather for their identification. We also thank Mr J. E. Burris and Drs T. Akazawa, J. S. Bunt, R. H. Burris, I. R. Price and D. D. Randall for assistance in collecting, and Dr L. Muscatine for advice. This work was supported by grants from the National Science Foundation to the Scripps Institution of Oceanography for operation of the Alpha Helix Research Program, and by a grant from the National Science Foundation to the senior author.

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Heteroantagonism observed in mixed algal cultures

RUSSELL¹ has referred to the curious lack of interest shown by marine ecologists in species competition, especially in view of the suitability of existing cultural techniques for investigating this problem. Certainly much more experimental work using mixed cultures has been carried out by examining competition in planktonic algae²⁻⁷ than in benthic algae⁸. This is perhaps surprising in view of the wealth of information, particularly that derived from studies on the algal colonisation of cleared or new substrata, which suggests that competition factors also play active roles in benthic community structuring and may even, as deduced by Den Hartog⁹, influence zonation. Particularly interesting in this respect are the growing number of reports¹⁰⁻¹⁴ that polyphenols ('tannins') are actively liberated from the surfaces of brown algae, and that these compounds possess both antibacterial and antialgal properties¹⁵⁻²⁰. Indeed, the fucosan-vesicles or physodes commonly found in brown algal cells have been attributed the function of polyphenol storage²¹⁻²³. Although the antifouling properties of these secretions have been recognised^{7,16,17,19,20}, very little is known of their ecological influence beyond the surface of the host. Here I report on some observations of mixed cultures of the brown crustose alga *Ralfsia spongiocarpa* Batt. and the two crustose red algae *Porphyrodiscus simulans* Batt. and *Rhodophysema elegans* (Crouan frat. ex J. Ag.) Dixon.

Both the *Ralfsia* thalli and the two crustose red algae were collected from the rocky sides of low-tide-level pools at Peveril Port, Swanage, Dorset; laboratory cultures were established using unispores²⁴ and tetraspores, respectively. The abundance

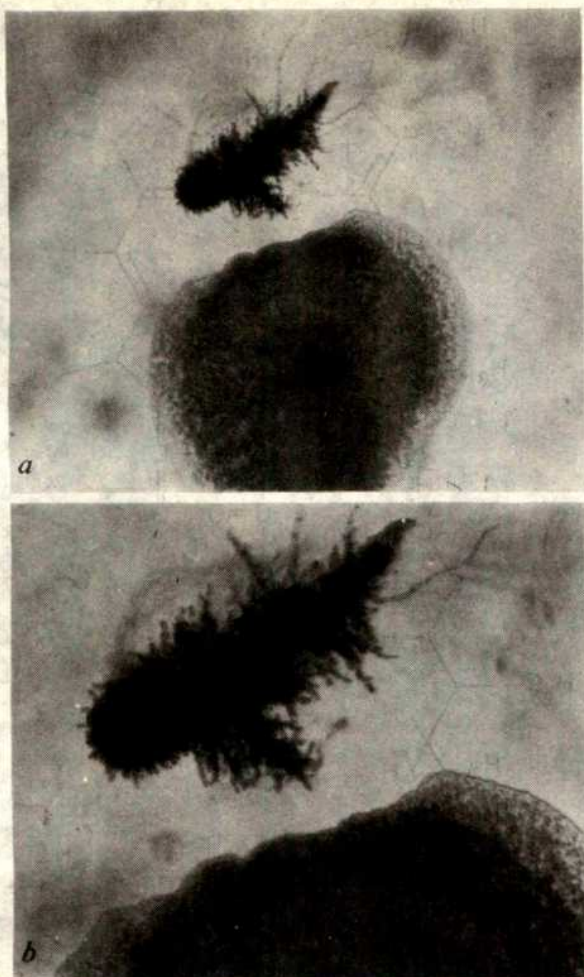


Fig. 1 a, Part of a 6-week-old cultured crust of *Rhodophysemma elegans* showing inhibition of horizontal growth on the side adjacent to a knot-filament of *Ralfsia spongiocarpa* ($\times 64$). b, Closeup of (a) ($\times 136$).

of the *Ralfsia* under the field conditions²⁵ suggested that the thalli had competitive advantages. Cultured crusts of the two red algae were later 'seeded' around their perimeter with small portions of cultured *Ralfsia* 'knot-filament' thalli (for a description of this phase in the life history see ref. 25). Care was taken to ensure that the *Ralfsia* thalli did not come into contact with the crusts but were allowed to form a weak attachment to the floor of the Petri dish a short distance away from them. 'Control' cultures were also set up in which an approximately equal amount of red algal 'knot-filament' growth was placed adjacent to cultured crusts of the same species. All cultures were placed at 10°C, 8–16-h day conditions using Gro-lux fluorescent tubes at

Fig. 2 Part of a 4-month-old cultured crust of *Porphyrodiscus simulans* showing inhibition of horizontal growth on the side adjacent to a knot-filament of *Ralfsia spongiocarpa* ($\times 45$).



an intensity of 1,000 lx. The culture medium was replenished each week.

Within approximately 10–14 d the growth of the two red algal crusts was markedly inhibited on the sides adjacent to the *Ralfsia* thalli (Figs 1 and 2). The broad monostromatic zone, typically produced in the other regions of the germlings and in the control cultures by outer, actively dividing meristematic cells, was absent and polystromatic crust formation was evident right up to the thallus margin (Fig. 3).

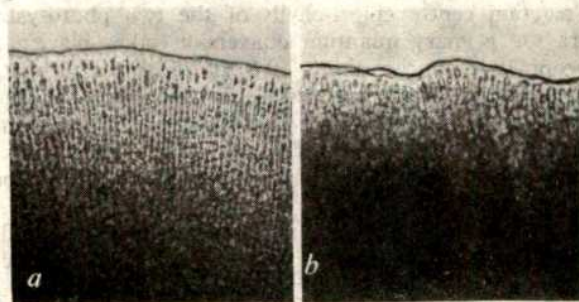


Fig. 3 a, Surface view of normal, actively growing margin of a cultured crust of *Porphyrodiscus simulans* ($\times 201$). b, Surface view of the margin of the same crust adjacent to the *Ralfsia* thalli ($\times 173$).

In view of the rapid nature of the response, it is unlikely that this growth inhibition effect was caused by differential nutrient uptake by the two adjoining algae. The evidence strongly suggests that an antibiotic, which inhibits the growth of the two red algae, is secreted into the culture medium by the *Ralfsia* thalli. Certainly, reports on the production of antibiotics in this genus are not new: Conover and Sieburth²⁶ reported that tannins secreted from *Ralfsia verrucosa* (Aresch.) J. Ag. in tidepools may be suppressing barnacle and mussel colonisation.

The possible involvement of antibiotics as controlling factors in crustose algal formations adds a new dimension to existing interpretations based predominantly on differential growth rates and/or selective grazer activity. It also bears important implications for the growth of erect thalloid species, the success of which will depend on the establishment and sustained growth of the basal attachment systems.

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Possible location of chlorophyll within chloroplast membranes

THE inner membranes of the chloroplasts of higher plants and green algae contain the photosynthetic apparatus for the conversion of solar energy to chemical energy¹. Light is absorbed primarily by the chlorophylls, most of which act in a light-collecting capacity to absorb and transfer energy to the reaction centre chlorophylls of the two photosystems where the primary quantum conversion takes place². The chlorophylls comprise about 10% of the mass of mature chloroplast membranes or 20% of the total lipid mass. Because of their key role in photosynthesis, a question of prime importance is the location of the chlorophylls in chloroplast membranes. Earlier proposals have been reviewed by Kreutz³.

A plausible model for membrane structure is the fluid lipid-protein mosaic where the membrane continuum consists of a lipid bilayer in which intrinsic proteins are embedded and to which extrinsic proteins are attached. Although intrinsic proteins may be highly hydrophobic they are amphipathic molecules with a hydrophilic region located at the membrane surface; in some cases the molecule may extend through the membrane and have two hydrophilic regions, one at each surface of the membrane⁴⁻⁷. There also seem to be two classes of lipids: fluid, mobile lipids which make up the matrix of the lipid domain; and fixed, boundary lipids, which consist of monomolecular layers attached to the hydrophobic shells of the penetrating intrinsic proteins^{8,9}.

Assuming the lipid-protein mosaic model to be valid for chloroplast membranes, the following reasons suggest that the bulk of the amphipathic chlorophyll molecules would be boundary lipids. If chlorophylls were located in the fluid lipid domain, the molecules would be free to move laterally in a random fashion through the membrane and the specific arrangement of the porphyrin rings essential for energy transfer would be impossible. This difficulty would not arise if the porphyrins were located in the protein region where ordered arrangement would be feasible. Indeed, it is likely that chlorophyll is attached to protein because two chlorophyll-protein complexes have been isolated from chloroplast membranes, following their solubilisation with sodium dodecyl sulphate. These two complexes make up some 70% of the intrinsic protein mass of mature chloroplast membranes: chlorophyll-protein complex 1, associated with photosystem 1, comprising some 20% and chlorophyll-protein complex 2, associated with photosystem 2, some 50% of the intrinsic protein^{10,11}. Moreover, it seems likely that most of the chlorophyll is attached to these complexes *in vivo*.

As well as porphyrin-protein interaction, the phytol chains would be associated with the hydrophobic regions of the two major intrinsic proteins. In a detailed molecular model proposed for chloroplast membranes, Weier and Benson¹² placed phytol chains, together with the acyl chains of the other chloroplast membrane lipids, into the hydrophobic interiors of lipoprotein subunits. Although this model¹³, in which the membrane is composed entirely of a sheet of lipoprotein subunits, is no longer considered feasible¹⁴, it is possible to consider that the phytol chains will reside exclusively within the hydrophobic regions of the intrinsic proteins of the lipid-protein mosaic membrane. Alternatively, I propose that phytol could be associated with the hydrophobic exterior of the intrinsic proteins and thus chlorophyll would be part of the boundary lipids of the two chlorophyll-protein complexes. Less energy would be required to attach the phytol chains to the protein exterior than to bury them completely in the hydrophobic protein

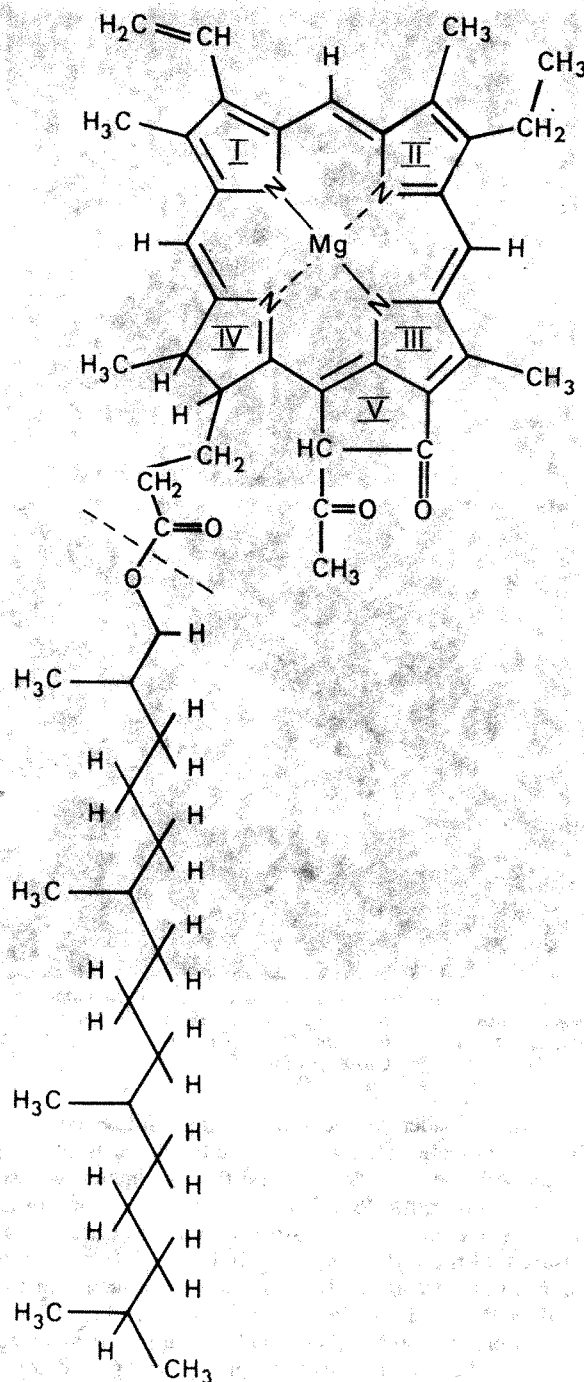


Fig. 1 Chlorophyll α .

interior. Further, anchoring the phytol chains at the protein exterior ensures that they will be perpendicular to the plane of the membrane and allows the porphyrin rings to be tilted with respect to the membrane surface.

Physical measurements indicate that the porphyrins are orientated at an angle of 48° to the membrane surface¹⁵. This orientation is analogous to that observed when chlorophyll is artificially incorporated into bilayers¹⁶⁻¹⁸. One edge of the square planar tetrapyrrole macrocycle, adjacent to phytol, is hydrophilic since it includes the cyclopentanone ring V and the carbonyl group of the propionic side chain of ring IV, to which phytol is attached; the Mg^{2+} also has hydrophilic properties (Fig. 1). This hydrophilic edge would interact at the aqueous membrane surface with the hydrophilic region of the intrinsic protein giving the porphyrin

ring the required tilt; the more hydrophobic portion of porphyrin would thus extend further into the hydrophobic protein region of the membrane. A schematic diagram is shown in Fig. 2 with chlorophyll molecules surrounding an intrinsic protein; additional lipids would be required to complete the boundary lipid monolayer. The necessary but undefined aggregations of the porphyrins would be possible in this model, and it would be expected in such arrays that the chlorophyll molecules will occur in several environments arising from interactions with other chlorophyll molecules, thus giving rise to the complexity of the *in vivo* spectra^{19,20}.

An additional reason for placing the light-harvesting chlorophylls as boundary lipids is evident when one considers the lipid content of chloroplast membranes. If intrinsic proteins are to be covered with boundary lipids as much as 35% of the total lipid molecules of chloroplast membranes may need to be immobilised. At least 30% of the total lipid mass consists of functional lipids, such as the chlorophylls, carotenoids and plastoquinones, which are not involved in formation of the bilayer. Obviously it is advantageous to use the chlorophylls, and probably also some carotenoids, as boundary lipids in order to leave sufficient lipids to form the fluid matrix.

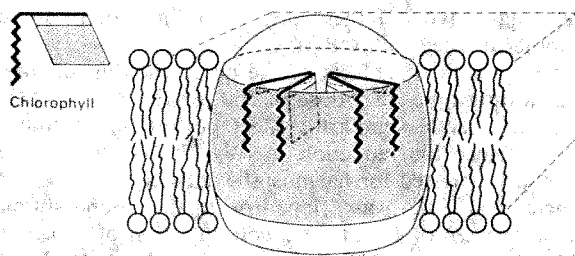


Fig. 2 Schematic cross section of a chloroplast membrane showing an intrinsic protein spanning the membrane, with hydrophilic regions located at the membrane surfaces and a hydrophobic portion (shaded) embedded within the nonpolar interior of the lipid bilayer. The chlorophyll molecules (shown on the left, with the hydrophobic portion of the porphyrin ring shaded) are part of the boundary lipid of a chlorophyll-protein complex. The phytol chains are perpendicular to the membrane surface in close interaction with the outside perimeter of the hydrophobic region of the intrinsic protein, and thus in contact on one side with protein and on the other side, with the acyl chains of the fluid lipid matrix. In contrast, the porphyrin rings are buried within the protein; the hydrophilic edge of the porphyrin ring is located at the membrane surface and the hydrophobic part is buried within the hydrophobic interior of the intrinsic complex.

If the chlorophylls are boundary lipids of the two major chloroplast intrinsic proteins as postulated, the porphyrins would be located towards the external or internal surface of the membrane. It is unlikely that the chlorophylls of an individual pigment complex would be divided between the outer and inner surfaces since this would markedly decrease the efficiency of light trapping. Briantais *et al.*²¹ favour the view that the porphyrin rings of the two photosystems are located on opposite sides of the membrane, with photosystem I at the external surface. On the other hand, Radunz²², using an antiserum monospecific to chlorophyll α , showed that part of the chlorophyll α of both photosystems was accessible to antibodies. Thus, a definitive answer to the question is not yet available. An important consequence, however, of chlorophyll being a boundary lipid is that the chlorophyll molecules will not be uniformly distributed over the membrane; rather they will occur only where the intrinsic proteins are located.

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Prostaglandins as haemostatic agents

It has been reported that prostaglandin endoperoxides (PGG₂ and PGH₂), possible intermediates in the biosynthesis of PGE₂ and PGF_{2α} from arachidonic acid, may be mediators of platelet aggregation¹⁻³. Kloeze reported⁴ that neither PGE₂ nor PGF_{2α} cause platelet aggregation or ADP release, and Willis⁵ stated that prostaglandins themselves cannot induce platelet aggregation. But we have found that three synthetic prostaglandins, Wy-16, 991, Wy-17, 185 and Wy-17, 186, induce platelet aggregation *in vitro*, and two of them, Wy-17, 185 and Wy-17, 186, significantly shorten the Lee White clotting time in rats. The most active of the three, Wy-17, 186, exerts a haemostatic effect on a bleeding wound surface.

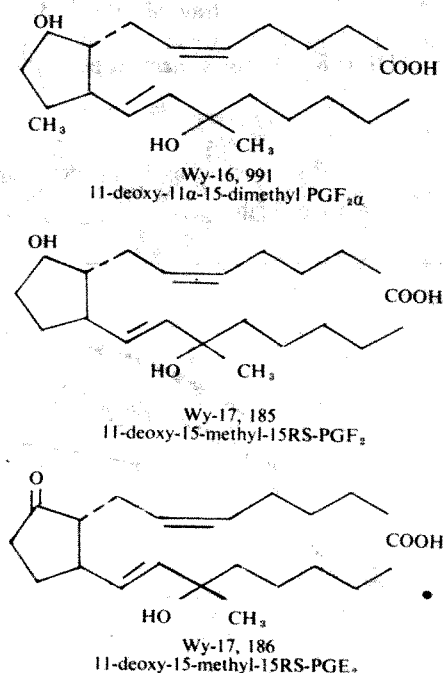


Table 1 Effect of prostaglandins on Lee White clotting time of fasted male rats

Compound	Dose ($\mu\text{g kg}^{-1}$)	No. of rats	Mean Lee White clotting time (s \pm standard error)		5 min
			1 min	No. of rats	
Control	—	8	341.2 \pm 13.1	7	340.7 \pm 13.6
Wy-17, 186	250	11	330.0 \pm 22.9	9	296.7 \pm 18.5
			NS		NS
Control	—	9	378.3 \pm 12.4	8	356.3 \pm 25.1
Wy-17, 186	500	9	281.7 \pm 12.4	7	242.1 \pm 24.9
			$P < 0.01$		$P < 0.01$
Control	—	9	393 \pm 10.9	8	401 \pm 8.4
Wy-17, 18	500	11	358 \pm 10.9	8	363 \pm 9.0
			$P < 0.05$		$P < 0.05$
Control	—	10	405.0 \pm 21.1	8	373.1 \pm 24.9
Wy-17, 185	250	10	363.8 \pm 14.2	5	369.0 \pm 22.2
			NS		NS
Control	—	8	433.1 \pm 9.8	5	435.0 \pm 9.2
Wy-16, 991	500	8	405.0 \pm 12.5	6	412.5 \pm 16.3
			NS		NS

Human blood was collected in siliconised 50 ml Vacutainers (Becton and Dickinson) fitted with siliconised 18 gauge needles using 3.8% sodium citrate as the anticoagulant (9 parts of blood to 1 part of sodium citrate). Platelet-rich plasma (PRP) was separated from the red blood cells and the effect of the prostaglandins on platelet aggregation was studied with a Payton Aggregation Module as described previously⁶.

To test the effect of these compounds on blood coagulability, groups of ether-anaesthetised male rats were given intravenous injections of the prostaglandin in physiological saline or saline control solution. Blood was obtained by cardiac puncture for the determination of the Lee White whole blood clotting time either 1 or 5 min after injection.

The haemostatic activity at a bleeding wound surface was determined by either exposing the femoral vein of Nembutal-anaesthetised rats and making a standardised nick (1–2 mm) in the vein with a lancet, to yield a free flow of blood, or opening the abdominal cavity to expose the mesenteric matrix of small blood vessels and making a 1–2-cm cut across these vessels with a scalpel. A 200 or 400 $\mu\text{g ml}^{-1}$ solution of the prostaglandin in physiological saline, or plain saline, was dripped on to the bleeding surface at a rate of about 1 drop per 5 s, and the time required for the cessation of bleeding was measured. The prostaglandins used were synthesised by Drs D. Strike and W. Kao.

A typical plot of the platelet aggregation induced in human PRP by these prostaglandins (3 $\mu\text{g ml}^{-1}$) compared with that induced by ADP (Fig. 1) shows them to be slightly less active

than ADP. Maximal platelet aggregating activities of Wy-17, 185 and Wy-17, 186 at 26 μM are inhibited 50% by 0.033 μM and 0.092 μM PGE₁, respectively. It is interesting that the aggregating activity of these prostaglandins can be inhibited by another prostaglandin, PGE₁.

Table 1 shows the effects of the prostaglandins on the Lee White clotting time. In a dose of 500 $\mu\text{g kg}^{-1}$ Wy-17, 185 and Wy-17, 186 had a significant effect both 1 and 5 min after injection. Since the reduction by Wy-17, 186 was somewhat greater, it was tested for haemostatic activity *in vivo*.

When Wy-17, 186 was dripped on to the wound surface of the femoral vein of 10 rats at a concentration of 200 $\mu\text{g ml}^{-1}$, the bleeding time was 77.8 \pm 5.6 s compared with 97.4 \pm 6.2 s for 10 controls. This bleeding time reduction was significant ($P < 0.05$). When a 400 $\mu\text{g ml}^{-1}$ concentration of Wy-17, 186 was instilled over the bleeding mesenteric vascular bed, the bleeding time for seven rats was 174 \pm 16.9 s, compared with 395 \pm 25 s for six controls. This reduction in bleeding time was significant ($P < 0.001$).

Apart from the endoperoxides, the activity of the stable prostaglandins reported here represents a unique example of prostaglandins capable of inducing platelet aggregation. Furthermore, Wy-17, 185 and Wy-17, 186 can shorten the Lee White clotting time, and Wy-17, 186 significantly shortens the bleeding time from a wound surface. The control of bleeding from a wound surface is a first step in the healing process. A potential application of the activity of this haemostatic prostaglandin may be in the prevention of excessive postsurgical bleeding, or in helping to control oozing of blood from a wound surface during surgery.

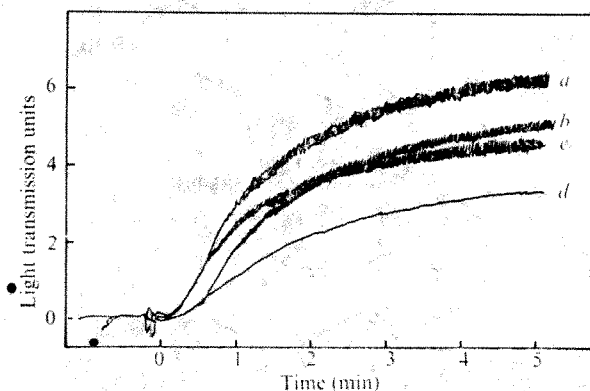
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Fig. 1 Effect of prostaglandins on platelet aggregation (a) 8.5 μM Wy-17, 185; (b) 8.5 μM Wy-17, 186; (c) 8.1 μM Wy-16, 991; (d) 3 μM ADP used as a comparative reference.



'microcrystalline' and which is not easily detectable by DTA, becomes dissolved and leaves behind a coarser residue which gives a more pronounced DTA inversion peak.

Talbot⁶ states that under the abnormally severe conditions of prolonged dry grinding employed by Sakabe⁷, quartz can be converted to vitreous silica. Our evidence, however, does not support that idea.

The transformation of quartz to vitreous silica during grinding depends on whether conditions are such as to cause melting of the quartz. High temperatures and pressures during grinding are often vaguely discussed in terms of adiabatic compression, friction, electrical or other effects, but it seems⁸⁻¹⁰ that pressures of only a few tens of kilobars are produced during grinding.

Wackerle¹¹ demonstrated the conversion of quartz to vitreous silica using explosive driving systems to generate shock pressures in excess of 383 kbar. It seems unlikely that pressures of that magnitude would be produced during the process of grinding. Friction might produce temperatures of the order of 1,500°C (required to melt quartz) but only for very short periods because the average temperature of a mill during running is typically no more than 50°C; but the melting of quartz is quite slow¹² and it can be considerably overheated without melting if the temperature is rising rapidly. Thus, it seems that even under prolonged dry grinding, conditions cannot result in the transformation of quartz into vitreous silica.

Sakabe⁷ stressed the importance of the very small particles which adhere to the larger ones and he apparently never concluded that vitreous silica was produced from quartz during grinding.

One of the great difficulties of applying the concept of a 'Beilby layer' to the grinding of substances, was encountered by Clelland¹, who found that vitreous silica itself exhibited an initially high solubility when ground to a powder. The production of a skin of vitreous material over a material already vitreous does not explain satisfactorily an initial high solubility. The explanation seems to lie either in the formation of very small adherent particles, or grains within each particle, or perhaps more probably is caused by absorption of atmospheric moisture during grinding, resulting in hydrated layers. Both quartz and vitreous silica absorb atmospheric moisture during grinding¹³ and that effect seems to have been overlooked in relation to such properties as solubility characteristics or density of the powder; many researches either do not mention drying procedures, or else have used inadequate drying procedures which do not provide the temperatures of the order of 1,000°C required to liberate the combined water.

We conclude that quartz is converted during grinding into a microcrystalline form not readily detectable by differential thermal analysis. Processes such as sedimentation or etching in hydrofluoric acid, which result in the removal of the finest particles, leave behind coarser residues which behave more like the original quartz and give a more pronounced inversion on DTA curves.

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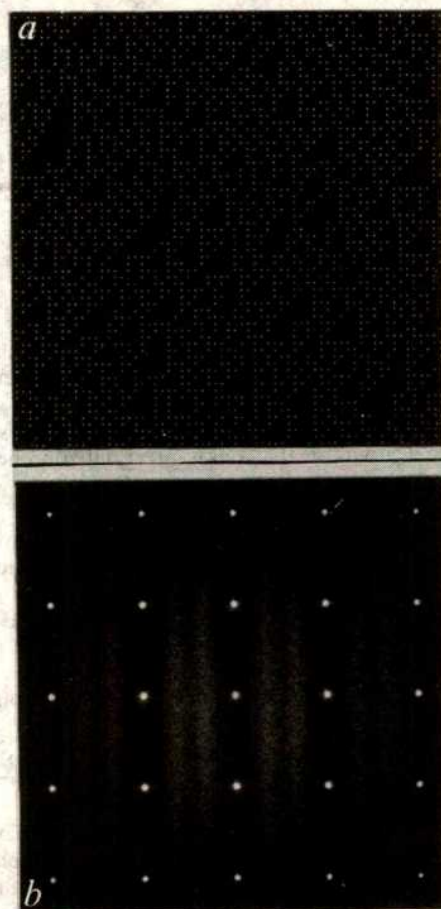
Short-range order in crystals

DURING recent studies of computer-grown crystals evidence has come to light that could possibly account for an inconsistency which has hitherto existed in the interpretation of the X-ray diffraction patterns of some real crystals which contain short-range order.

It has become increasingly evident that many crystalline materials ranging from minerals to organic molecular crystals contain some kind of short-range order. In many the interatomic forces which give rise to short-range order (either at crystallisation or subsequently through the process of diffusion) are relatively long-range, but in organic molecular crystals the forces of interaction between molecular species are short-range van der Waals' forces typically acting over only a few Ångströms. In such cases it has seemed inconsistent when the short-range order as observed by X-ray diffuse scattering apparently indicates interactions over much greater distances.

For example, during its phase transition acenaphthylene¹ assumes a structure tending to repeat every 42 Å along the *c* axis although the dimension of its unit cell at room temperatures is only 14 Å and the closely packed molecular layers are only 7 Å apart. One-dimensional models of crystal disorder^{2,3} suggest that to explain such a pseudo-tripling of the cell it is necessary to consider interactions between second and possibly third

Fig. 1 *a*, Sample array; *b*, optical diffraction pattern produced by this array.



nearest neighbouring cells, that is interactions in the range 30–40 Å.

But a two-dimensional model of crystal disorder has been developed³ and more recent studies of this model have shown that it is possible to produce a pseudo-tripling of a cell repeat in one direction when only nearest-neighbour interactions are involved.

The array shown in Fig. 1a is a small representative portion of a much larger array (500 × 500 lattice points) which was computer-grown with the model described in ref. 3. The occurrence of the two molecular species A (represented by a hole) and B (represented by a blank) at a particular lattice site is decided by consulting the nearest lattice point above and the nearest to the left. For a given combination of the species at these two lattice points (there are four: A^A, A^B, B^A, B^B) there is a constant probability that the lattice point in question is an A. In the particular example shown the probability that an A occurs after A^A is 0.0, the probability that an A occurs after B^B is 0.2, the probability that an A occurs after an A^B is 0.8, and the probability that an A occurs after B^A is 1.0.

The optical diffraction pattern of this array (Fig. 1b) has a peak in the diffuse diffraction profile at about 1/3 and 2/3 of the cell repeat indicating a tendency to a tripling of the repeat in the lattice of Fig. 1a, though this is not readily apparent simply by viewing the lattice. I emphasise that such a diffraction profile is not possible with a one-dimensional model of disorder if only nearest-neighbour interactions are involved.

It seems likely that, in real crystals where interactions in three dimensions must be considered, even more dramatic effects may be possible. Effects such as those shown by acenaphthylene may, therefore, not need the consideration of non-nearest-neighbour interactions: this possibility is being investigated by extending the present two-dimensional models to three dimensions.

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Gynodioecy in an animal

GYNODIOECY, a breeding system in which the population consists of hermaphrodites and females only, is a well known botanical phenomenon with a literature dating back at least to Darwin¹. It has never previously been reported in an animal.

A population of the sea anemone *Epiactis prolifera* Verrill, 1869, living in the rocky intertidal zone near Bodega Marine Laboratory, Sonoma County, California, was studied from April 1970 to March 1972. Of 269 animals from monthly collections examined histologically, 31 were sterile, 138 were female, and 100 were hermaphroditic (Fig. 1). This pattern of gynodioecy persisted all year round. Fertile anemones ranged from 5.8 to 35.7 mm in basal diameter. As an effort was made to include individuals of all sizes in each collection, the largest and smallest animals were represented in excess of their relative proportions in the population.

E. prolifera broods its young externally, one of 16 actinian species known to do so². Females and hermaphrodites spawn relatively few large yolky eggs which soon adhere to the parent's ectoderm, where they develop directly into juvenile anemones. When sufficiently large, juveniles move from the parent on to the surrounding substrate (D.F.D., unpublished).

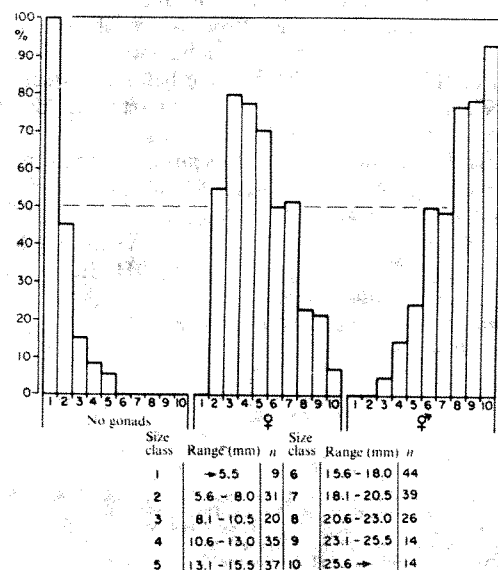
Some ideas from the botanical literature are relevant to an analysis of the breeding system of *E. prolifera*, but that part consisting of genetic analyses and models^{1,3–8} is not. Among gynodioecious plants which have been examined, individuals are genetically either hermaphroditic or female. In *E. prolifera* it seems that sex determination is virtually identical in all individuals so that animals begin their reproductive lives as females, and most that survive sufficiently long become hermaphrodites. Thus gynodioecy in *E. prolifera* is serial and developmental, whereas in plants it is fixed and genetic.

A major advantage of gynodioecy—its ability to adjust the degree of heterozygosity in a population^{3,4,9}—obtains only if the species is at least potentially self fertile. There is no reason to dismiss this possibility in *E. prolifera*, where ripe ova and spermatozoa frequently occur in the same animal simultaneously (D.F.D., unpublished). If hermaphroditic individuals were facultatively self fertile, advantageous characters, including those which enabled them to survive sufficiently long to develop testes, could be fixed in the genome of the population by homozygosity. Under favourable environmental conditions, longevity would increase, raising the proportion of hermaphrodites in the population and thereby the potential for inbreeding. When conditions were such that few anemones survived long enough to develop testes, inbreeding would decline, thus increasing the proportion of heterozygous offspring with greater genetic flexibility to meet the stressful conditions.

Genetic factors allowing animals to survive long enough to develop testes and producing gynodioecy in *E. prolifera* are favoured numerically, even if selfing is impossible. Offspring of females receive half their genes from hermaphrodites; those of hermaphrodites receive all of theirs from hermaphroditic parents, one if by selfing and two if by crossing. Thus the genetic contribution to the next generation from hermaphrodites, no matter how few, cannot be less than 50%. It is usually greater because hermaphrodites will have already produced offspring as females, and because larger anemones brood more young (D.F.D., unpublished).

It is crucial to the species' success that the prevalence of egg-producing individuals in the population be as great as possible, for fecundity of *E. prolifera* is restricted by brood space. The strategy of having barely enough males to fertilise the ova produced by a population consisting largely

Fig. 1 The relationship between size and gonadal state of 269 *E. prolifera* collected approximately monthly over a period of 2 yr. Size classes are based on pedal disk diameter at the time of collection.



Effects of temperature and pressure on short term storage of platelets

NONE of the methods suggested so far to improve the storage life of platelets has proved entirely satisfactory¹⁻⁵. Storage at 22°C is the most accepted method³, and other suggestions include the use of chemical additives such as prostaglandin E₁ (ref. 5) or freezing techniques⁷⁻⁹. Studies involving a controlled rate of freezing and thawing in the presence of dimethyl sulphoxide have been encouraging¹⁰⁻¹², but a better method is desirable as the need for platelets is increasing rapidly. An improved method of storage might be developed if the normal discoid shape could be maintained. Furthermore, if the change in shape that occurs when platelets are cooled is accompanied by an increase in volume, as has been suggested¹³⁻¹⁵, or if the strength of the bonds maintaining the secondary and tertiary structure of the protein of the platelets is affected significantly by temperature or pressure, the application of sufficient pressure might shift the equilibrium towards a conformation favouring the discoid form. We have now found that pressure (2,500–9,000 pound inch⁻²) (17.24–61.65 N m⁻²) prevents to a considerable degree these changes in shape, and restores the shape of platelets in concentrates that have been kept at 2°C and atmospheric pressure for 1–2 h.

We first monitored the changes in shape using a water-jacketed high pressure optical cell in a Cary 14 spectrophotometer and samples of human and bovine blood platelet-rich plasma and platelet concentrate when pressure and temperature were varied. (Human platelet concentrates, from the Community Blood Bank of Kansas City, were prepared by collecting 500 ml of whole blood in a Fenwall GA-35 transfer pack containing 70 ml of acid citrate dextrose solution. Samples were centrifuged at 3,000g for 3 min, and the supernatant platelet-rich plasma was centrifuged at 5,000g for 5 min. The platelets were then resuspended in about 30 ml of plasma.) When low temperature studies were conducted a stream of nitrogen was passed through the cell compartment to prevent water condensation. Cooling consistently caused an increase in the absorbance which was reversed when pressure was applied to the cooled sample. A typical

Fig. 1 Absorbance at 620 nm of a platelet concentrate diluted 1:2 with saline solution as a function of applied pressure. O, Absorbance values measured 5 min after application of the designated pressure; □, absorbance corrected for the effect of pressure at room temperature. The sample is ~24 h old.

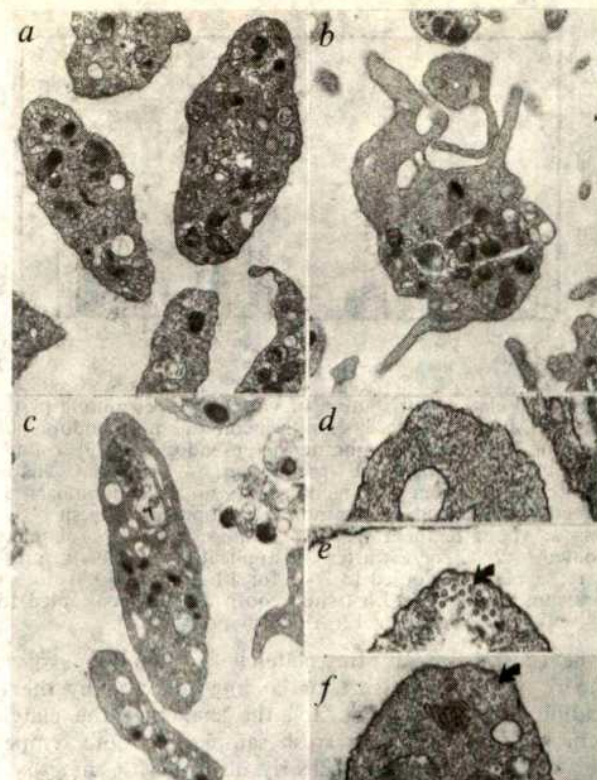
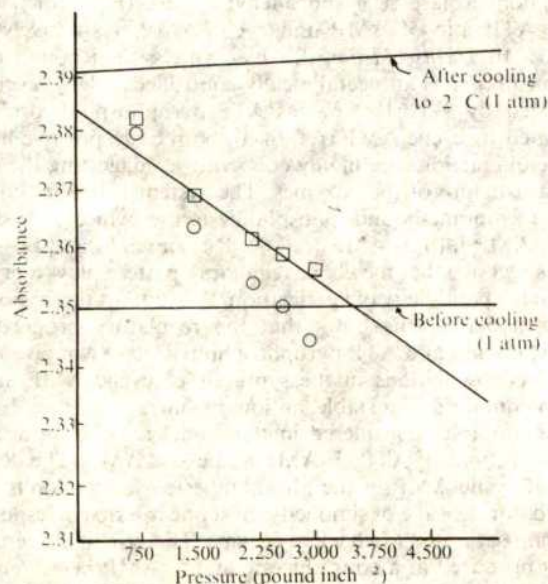


Fig. 2 Electron micrographs of platelets showing: *a*, typical discoid profile at room temperature and 1 atm of pressure; *b*, the numerous pseudopodial extensions and rounded form at low (2°C) temperature and 1 atm pressure, and *c*, discoidal forms at low temperature accompanied by pressure. (*a-c*, $\times 13,400$). Micrographs at higher magnification of the margin of platelets show (*d*) the absence of the marginal band of microtubules at low temperature with pressure, (*e*) the marginal band of microtubules (arrow) at room temperature and 1 atm pressure and (*f*) the band (arrow) at room temperature and 5,000 pounds inch⁻² (34.48 N m⁻²) pressure. (*d-f*, $\times 23,830$).

example of the data thus obtained is shown in Fig. 1. In several cases, pressure decreased absorbance to the original value (that is, the value at room temperature), but, in some cases, pressure seemed to cause only a slight decrease in absorbance. In the same apparatus, pressure followed by cooling caused a smaller increase in absorbance than did cooling alone.

For verification, and as an independent check on these results, we also used transmission electron microscopy. A high pressure cell with a dual chamber (a modified form of that described in ref. 16) was used to fix the platelets under high pressure at a controlled temperature. Suspensions of platelet concentrate were maintained in one of the chambers, and a solution of 5% glutaraldehyde with s-collidine buffer was kept in the other. When temperature, time and pressure were appropriate, the fixative was mixed with the platelet suspension to give chemical fixing in the desired conditions.

Figure 2 shows typical results obtained when a batch of platelet concentrate was examined under four conditions. At room temperature the platelets were discoid with profiles in thin sections ranging from circular to elongated ellipsoid. Figure 2*a* shows a typical elongated profile of a fresh platelet. Beneath the generally smooth contour of the surface membrane was a marginal band of microtubules circumscribing the uniformly distributed vesicles and granules in the cytoplasm (Fig. 2*e*). When cooled, virtually all platelets become rounded, with irregular surface contours associated with the presence of numerous fine pseudopodia (Fig. 2*b*). The marginal band of microtubules was lacking and the membranous organelles and granules were grouped together

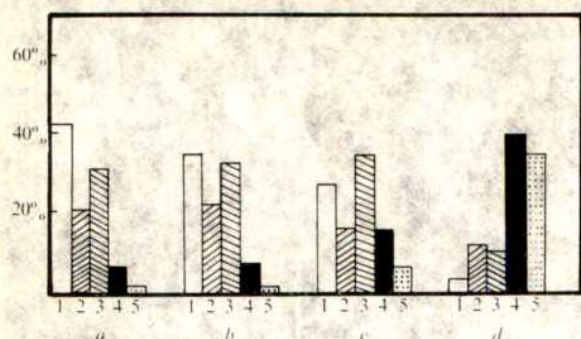


Fig. 3 Relative distribution of various shapes among platelets. 1, Definitely discoid, smooth membrane no pseudopodia; 2, round, smooth membrane no pseudopodia; 3, round, smooth membrane, one pseudopod; 4, irregular shape (caused by irregular membrane), no pseudopodia; 5, irregular shape and one or more pseudopodia; a, fresh platelets at room temperature and 1 atm pressure; b, platelets cooled to 2° C for 1 h after the application of 350 atm pressure; c, platelets cooled to 2° C for 1 h and then 350 atm of pressure applied for 1 h (while cool); d, platelets cooled to 2° C for 1 h at 1 atm pressure.

in the central area of the platelets. In conditions of low temperature with pressure or cooling followed by the application of pressure (Fig. 2c), the shapes of the platelets resembled those found in fresh samples at room temperature. Many platelets were clearly discoid with smooth surfaces and uniformly distributed organelles and granules. These discoid platelets had no marginal band of microtubules (Fig. 2d), although some were found in the central part of these platelets among the membranous organelles. Thus, microtubules do not seem to be essential to the maintenance of the discoidal shape of platelets under these conditions.

In an attempt to quantitate our results, we took photographs at low magnification and counted the platelets in each of five arbitrarily chosen categories based on shape and structure. The categories and their relative populations are presented in Fig. 3. About 93% of our fresh platelets fitted categories 1, 2 and 3, which were characteristic of normal fresh platelets. Treatment at 2° C for 1 h resulted in 25% platelets in categories 1, 2 and 3, and 75% in categories 4 and 5. More than 90% of the platelets cooled under pressure for 1 h fitted categories 1, 2 and 3, while 78% of those cooled for 1 h and then pressurised while cold for 1 h fitted categories 1, 2 and 3, with 22% in categories 4 and 5. Thus, it seems that pressure prevented the change in shape that typically occurs when platelets are cooled. Figure 3d indicates that pressure was less effective after cooling than before cooling.

In discoid cells maintained at low temperature under pressure there was no marginal band of microtubules (Fig. 2d) in contrast to platelets kept at room temperature and atmospheric pressure (Fig. 2e). To evaluate this further, platelets were fixed at room temperature under pressure, and examined with the electron microscope. In all cases, there was a marginal band of microtubules (Fig. 2f) indicating that, contrary to many suggestions¹⁷⁻²⁰ pressure alone in the ranges investigated did not cause disaggregation of the microtubules. Similar findings were obtained with microtubules from various nerve cells using the equipment described here²¹. The marginal band was generally not as clearly segregated into discrete bundles in these conditions as their counterparts at a pressure of 1 atm.

The fact that pressure definitely moderates the morphological changes caused by cooling, indicates that the use of physical methods (either alone or in conjunction with other methods) may lead to improved storage procedures for blood platelets. The measurement of some viability parameters of platelet samples subjected to various conditions of pressure and temperature is currently in progress.

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Mechanism for oscillatory synthesis of cyclic AMP in *Dictyostelium discoideum*

CELLS of the slime mould *Dictyostelium discoideum* aggregate around centres into multicellular fruiting bodies as a chemotactic response to cyclic AMP (refs 1 and 2). The wave-like character of this phenomenon suggests the release of cyclic AMP pulses by the founder cells^{3,4}. In this respect, metabolic oscillations controlled by cyclic AMP have been observed in suspension cultures of *D. discoideum* cells capable of forming aggregates⁵. We present here a mechanism for both these intracellular oscillations and the periodic release of chemotactic signal by aggregation centres.

The mechanism is based on the regulation of two enzymes directly involved in the synthesis of cyclic AMP, ATP pyrophosphohydrolase (E_1) and adenylyl cyclase (E_2), which transform ATP into 5' AMP and cyclic AMP, respectively⁶ (see Fig. 1). In *D. discoideum*, this bienzyme system bound to the cell membrane is autocatalytically controlled: adenylyl cyclase is activated by 5' AMP whereas ATP pyrophosphohydrolase is activated by cyclic AMP (ref. 6). In both cases positive heterotropic interactions are highly cooperative, suggesting the oligomeric structure of the enzymes. The system is further coupled to a membrane-bound phosphodiesterase which transforms cyclic AMP into 5' AMP (ref. 7). Rossomando and Sussman⁸ have suggested that the above regulatory pattern plays a primary role in the periodicity of aggregation. We support this hypothesis by a model which shows that the regulatory properties of adenylyl cyclase and ATP pyrophosphohydrolase can give rise to sustained oscillations in the synthesis of cyclic AMP around a non-equilibrium unstable stationary state.

The variables considered in the model are the intracellular concentrations of ATP, 5' AMP and cyclic AMP. The destruction of cyclic AMP in the phosphodiesterase reaction is represented, for the sake of simplicity, by a linear term corresponding to a non-saturated Michaelian enzyme. The system is open, ATP being produced at a constant rate and 5' AMP being removed in proportion to its concentration; the only sink taken into

account for cyclic AMP is that of the phosphodiesterase reaction. As this paper deals with the molecular basis of cyclic AMP oscillations at the cellular level and not with the process of slime mould aggregation, the effect of diffusion is not considered. It should be stressed, however, that oscillations in the intracellular cyclic AMP concentration may result, through transport of this metabolite across the cell membrane, in a periodic release of chemotactic factor into the extracellular medium. The cooperative kinetics of ATP pyrophosphohydrolase and adenylyl cyclase is described by the concerted model of Monod *et al.*⁸, assuming that these proteins are allosteric dimers and that the substrate and positive effectors bind exclusively to the active (R) state of each enzyme. In conditions of a quasi-stationary state for the enzymatic species, the time evolution of the metabolite concentrations is then given by the differential equations^{9,10}

$$\dot{\alpha} = v_1 - \frac{\epsilon_1 \alpha (1 + \alpha) (1 + \gamma)^2}{L_1 + (1 + \alpha)^2 (1 + \gamma)^2} - \frac{\epsilon_2 \alpha (1 + \alpha) (1 + \beta)^2}{L_2 + (1 + \alpha)^2 (1 + \beta)^2} \quad (1a)$$

$$\dot{\beta} = \frac{\epsilon_1 \alpha (1 + \alpha) (1 + \gamma)^2}{L_1 + (1 + \alpha)^2 (1 + \gamma)^2} - k_1 \beta + k_2 \gamma \quad (1b)$$

$$\dot{\gamma} = \frac{\epsilon_2 \alpha (1 + \alpha) (1 + \beta)^2}{L_2 + (1 + \alpha)^2 (1 + \beta)^2} - k_2 \gamma \quad (1c)$$

where α , β and γ denote, respectively, the normalised concentrations of ATP, 5' AMP and cyclic AMP; L_1 and L_2 , and ϵ_1 and ϵ_2 , are, respectively, the allosteric constants and the normalised maximum activities of enzymes E_1 and E_2 . The source term of the substrate is denoted v_1 , whereas k_1 and k_2 are the rate constants for the sink of the products β and γ . Concentrations and kinetic parameters (v_1 , ϵ_1 , ϵ_2) are normalised by division through the appropriate Michaelian or dissociation constants of enzyme-metabolite complexes^{9,10}.

The system described by equations (1a-c) admits a stationary state (α_0 , β_0 , γ_0) for $\dot{\alpha} = \dot{\beta} = \dot{\gamma} = 0$, that is, when the following relationships are satisfied:

$$\beta = v_1/k_1$$

$$\gamma = [\epsilon_2 \alpha (1 + \alpha) (1 + \beta)^2] / [k_2 (L_2 + (1 + \alpha)^2 (1 + \beta)^2)]$$

$$[\epsilon_1 \alpha (1 + \alpha) (1 + \gamma)^2] / [L_1 + (1 + \alpha)^2 (1 + \gamma)^2] = v_1 - k_2 \gamma \quad (2)$$

The dynamic behaviour of the model depends on the stability properties of the stationary state (α_0 , β_0 , γ_0). The latter have been investigated using a normal mode analysis¹¹ after graphical resolution of equations (2) on a digital computer. The results of the stability analysis are indicated in Fig. 2 as a function of

Fig. 1 Mechanism for the oscillatory synthesis of cyclic AMP in *D. discoideum*, based on the activation of ATP pyrophosphohydrolase (E_1) by cyclic AMP and of adenylyl cyclase (E_2) by 5' AMP. Constants k_1 and k_2 relate to the 5' nucleotidase⁶ and phosphodiesterase reactions (see text).

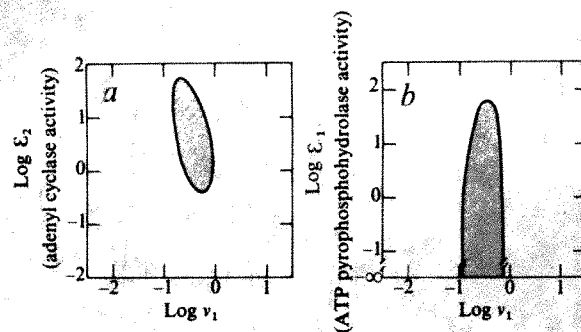
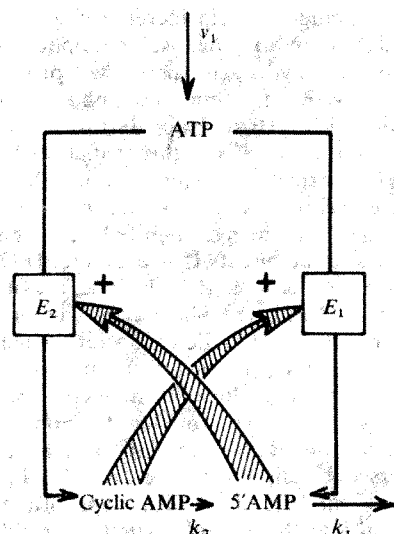


Fig. 2 Domain of sustained oscillations (shaded area) as a function of ATP injection rate (v_1), adenylyl cyclase and ATP pyrophosphohydrolase activity. The diagrams are established for $L_1 = L_2 = 10^6$, $k_1 = k_2 = 0.1 \text{ s}^{-1}$, a , $\epsilon_1 = 10 \text{ s}^{-1}$; b , $\epsilon_2 = 10 \text{ s}^{-1}$. Outside the shaded area the system evolves towards a steady state corresponding to a constant intracellular cyclic AMP level and, in the case of a linear sink to the extracellular medium, to steady release of chemotactic signal.

adenylyl cyclase and ATP pyrophosphohydrolase activity, for varying ATP injection rate. Each diagram is divided into two domains: outside the dashed region, the system evolves toward a stable steady state, whereas in the shaded domain of temporal dissipative structures^{11,12}, the system undergoes sustained oscillations around a non-equilibrium unstable stationary state with unique amplitude and frequency (Fig. 3). The period of the phenomenon is extremely stable with regard to enzymatic activities and substrate injection rate; it varies by less than a factor of two in the oscillatory range of parameter v_1 and a similar variation obtains for ϵ_1 and ϵ_2 . The oscillatory domain of each enzyme activity extends over more than two orders of magnitude, in contrast to the narrow unstable range observed for the substrate input (Fig. 2). Increasing ϵ_1 , ϵ_2 or v_1 lowers the period, whereas the amplitude of the oscillations passes through a maximum with respect to v_1 and ϵ_2 and diminishes with increasing ϵ_1 from zero. The role of enzyme cooperativity in the mechanism of instability is illustrated by the fact that the oscillatory domain corresponds to large values for the allosteric constants L_1 and L_2 , of the order of 10^6 . Predictions as to the order of actual metabolite concentrations in the course of oscillations are beyond the scope of the present study and require a precise knowledge of *in situ* kinetic parameters of membrane enzymes E_1 , E_2 .

Despite its relative simplicity, the model yields a qualitative agreement with a number of experimental facts. The waveform of the oscillations in Fig. 3 accounts well for the pulsatory production of cyclic AMP observed in slime mould aggregation; on the other hand, the restricted range of the period corresponds quantitatively to the observation that founder cells in *D. discoideum* oscillate with a period of 3–5 min⁴. Both the pulsatory nature of the oscillations and the stability of the period result from the allosteric properties of the enzymes involved in the instability mechanism, as previously shown^{10,13} for phosphofructokinase in the case of glycolytic oscillations¹⁴.

The model also accounts for some of the experiments performed in cell suspensions. It should be stressed that these experiments⁵ suggest the existence of a metabolic oscillator controlled by cyclic AMP and the independent operation of an amplification mechanism, whose nature is still not clear, for the relay of cyclic AMP pulses^{3,4}. The latter process, linked to the membrane receptor for cyclic AMP, allows control of intracellular cyclic AMP synthesis by extracellular cyclic AMP. The response coupled to the cyclic AMP receptor is not considered in the model of Fig. 1, which relates only to the multi-enzyme oscillator, but provision is made for the model oscillatory system to react to externally added cyclic AMP as described below.

The interaction of cyclic AMP with the oscillating system has been studied experimentally in suspended cells by investigating the response to pulses or continuous injection of this metabolite.

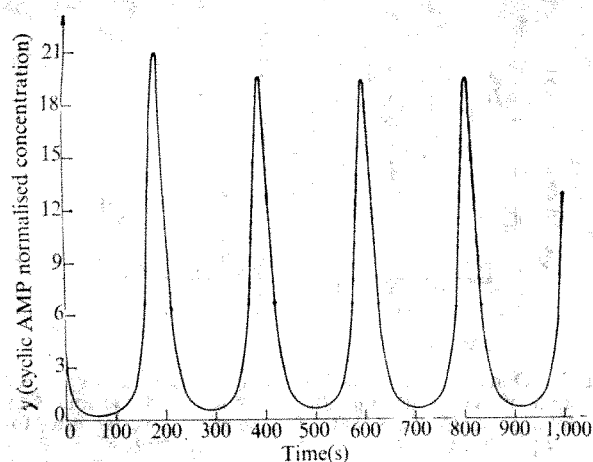


Fig. 3 Sustained oscillations of cyclic AMP. The curve is obtained by integration of equations (1a-c) using the Continuous System Modelling Program on an IBM 370-165 computer, for the following set of arbitrary values of the parameters: $\epsilon_1 = 0.4 \text{ s}^{-1}$, $\epsilon_2 = 10 \text{ s}^{-1}$, $k_1 = K_2 = 0.1 \text{ s}^{-1}$, $v_1 = 0.5 \text{ s}^{-1}$, $L_1 = L_2 = 10^6$. Initial conditions: $\alpha = 8$, $B = 4$, $\gamma = 4$. The normalised concentrations of ATP (α) and 5' AMP (β), (not shown) oscillate in the range 15-67 and 0.6-18, respectively. Periodic variation of intracellular cyclic AMP may result in a periodic release of chemotactic factor as a result of transport across the cell membrane.

Continuous addition of cyclic AMP does not modify the period of the phenomenon until injection rates are reached which extinguish the oscillations⁵. Correspondingly, in the model, addition of a constant source term in the kinetic equation (1c) for γ changes the period by less than 5% below the value 0.1 s^{-1} in the case considered in Fig. 3; above this threshold, oscillations disappear as the system evolves towards a steady state. As to the effect of discontinuous cyclic AMP addition, computer simulations of the model indicate that pulses of γ in the first part of the period after a peak of cyclic AMP delay the oscillations, with a maximum effect near the middle of the period; a small advance in the phase is observed when the pulse is applied in the second part of the period. These results and the fact that the minimum phase shift is found in the vicinity of a peak agree with experimental observations⁵. The differential sensitivity of the adenyl cyclase oscillator to cyclic AMP suggests a molecular basis for the refractory period observed in the chemotactic signalling process during aggregation³⁻⁵.

Two further remarks can be made about the experiments in suspended cells of *Dictyostelium*. The fact that 5' AMP does not interact with periodic behaviour⁵, contrary to the predictions of the model, could result from the lack of a membrane receptor for this metabolite. On the other hand, the model suggests that oscillations observed in the cytochrome chain⁵ result from the periodic variation of ATP in the adenyl cyclase system.

As a result of the formation of 5' AMP from cyclic AMP in the phosphodiesterase reaction, oscillations still arise in the absence of ATP pyrophosphohydrolase as indicated in Fig. 2. Thus periodic behaviour may result from the regulatory properties of adenyl cyclase only, although the reverse is not true for the second enzyme. For $\epsilon_1 = 0$, the model yields a three-variable autocatalytic mechanism for glycolytic oscillations with activation of phosphofructokinase by AMP; α , β , and γ denote then the concentrations of ATP, AMP and ADP, respectively. This generalises the results previously obtained in a two-variable model¹⁰ for glycolytic oscillations and stresses the similarity between the oscillatory mechanism in glycolysis and cyclic AMP synthesis. No interference between these oscillatory phenomena can be expected in aggregating cells of *D. discoideum* since glycolytic periodicities in yeast and muscle are linked to the cooperative properties of phosphofructokinase^{10,11}, which is not allosteric in the slime mould¹⁵.

Non-oscillatory mutants of *D. discoideum*, such as *ap66* (ref. 4), and oscillatory mutants with altered periodic properties,

such as *Fr17* (ref. 16), presumably differ from the wild type in the regulatory properties of one of the enzymes involved in the unstable mechanism¹⁷. More specifically, the model suggests that non-oscillatory mutants may lack activation of adenyl cyclase by 5' AMP. Particularly relevant is the finding that the regulatory pattern observed in *D. discoideum* (Fig. 1) also obtains in *Polysphondylium violaceum*⁸. Oscillations with a period of 1.5 min have been observed during aggregation in this species¹⁸, suggesting that the chemotactic attractant in *P. violaceum* is a metabolite linked to the oscillating system.

At the supracellular level, some models for *D. discoideum* aggregation postulate the existence of an oscillatory source of cyclic AMP (ref. 19). The mechanism discussed here lends support to such a postulate. Moreover, equations 1a, b and c could be included, with the effect of diffusion, in a set of equations that describe the aggregating system²⁰. The model should then be extended to include a second mechanism for the relay of cyclic AMP pulses, linked to the membrane receptor for this metabolite. Wave-like aggregation should result.

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Ion movement across leukocyte plasma membrane and excitation of their metabolism

A PERTURBATION of the molecular organisation of the surface membrane of mammalian phagocytes leads to significant modifications of their metabolism and functions^{1,2}. These modifications include a rapid increase of oxygen uptake, of glucose oxidation by the hexose monophosphate pathway (HMP) and of hydrogen peroxide production¹⁻³; an enhanced turnover of membrane phospholipids⁴ and a degranulation with extrusion of lysosomal proteins from cells^{5,6}. The importance of this functional response of phagocytes is particularly evident when the alteration of their surface properties is caused by interaction with bacteria, which, on being engulfed, can be killed and digested by the toxic agents (for example, H_2O_2 , superoxide anions, cationic proteins and hydrolytic enzymes) produced and/or discharged into the phagocytic vacuole⁶⁻⁹.

The trigger of the metabolic excitation, which has puzzled several investigators in the past, has so far escaped identification. We have shown^{1,2} that concanavalin A (con A), both in solution and coupled to non-phagocytosable beads, induces a metabolic stimulation of leukocytes, which can be reversed by dislodging the cross-linking lectin from its membrane binding sites with appropriate sugars. We have suggested that the 'on-off' switch, operating at the level

of the leukocyte surface membrane, involves a process of association-dissociation of membrane protein subunits. The clustering of these subunits should lead to a local increase in membrane potential and/or permeability to ions^{1,10,11}. To test this hypothesis we have now studied the effect of lanthanum ion and some ionophores on the metabolism of polymorphonuclear leukocytes (PMNL). The rare earth cation has been shown to bind to cell membrane protein and to induce an ion redistribution across the membrane¹². The ionophores easily form complexes with alkali or alkali earth ions. The complexes freely diffuse across membranes, thereby mediating a passive transport of cations down their concentration gradient¹³.

The ionophores used were valinomycin and nigericin, which form lipid soluble complexes with monovalent cations^{13,14}; A23187, a carboxylic antibiotic which transfers Ca^{2+} and Mg^{2+} across biological membranes¹⁵; and X537A, another carboxylic antibiotic which binds and equilibrates both monovalent and divalent cations¹³. PMNL were isolated from peritoneal exudates of guinea pigs injected with sterile 1.2% caseinate 15–16 h earlier. Cells were suspended in either calcium-free Krebs-Ringer phosphate with 1.2 mM Mg^{2+} (KRP-Mg) or calcium-free Krebs-Ringer Tris with or without 1.2 mM Mg^{2+} (KRT-Mg and KRT, respectively). Tris buffer was used instead of phosphate to avoid formation of precipitates with lanthanum and calcium ions. Respiration of PMNL was monitored by means of a Clark-type oxygen electrode and glucose oxidation by counting $^{14}\text{CO}_2$ produced from labelled glucose¹. Stock solutions of the ionophores were prepared in dimethyl sulphoxide (DMSO), the final concentration of which in the cell suspensions never exceeded 0.5% (v/v).

About 2 min after the addition of lanthanum ions to PMNL suspended in KRT-Mg, there was a pronounced increase in cell respiration (Fig. 1). At the same time, the leukocytes underwent an activation of glucose oxidation which was measurable even at 10^{-5} M concentrations of La^{3+} (Fig. 2). The use of exogenous glucose labelled either in position 1 or 6 led to the conclusion that the only metabolic pathway activated was the HMP.

Also the addition of X537A to PMNL caused a very rapid and extensive enhancement in oxygen uptake (Fig. 1). Because of the response time of the oxygen probe used (90% in 10 s) and the time required to inject the ionophore into the cell suspension, we conclude that the onset of metabolic activation is almost immediate. On a concentra-

tion basis the stimulatory efficiency of X537A in KRP-Mg is far greater than that of valinomycin, nigericin or A23187. The stimulation of respiration by all these agents is not affected by 1 mM NaN_3 , thus ruling out an activation of the mitochondrial oxidative systems. In addition, the stimulation of oxidation of 1- ^{14}C -glucose, and not of 6- ^{14}C -glucose (Table 1), shows that the respiratory stimulation is linked to an increase in the rate of HMP.

As well as acting as ion carriers, the ionophores might exert their biological effects by a mechanism unrelated to the ion movement. At least for A23187 this possibility seems unlikely, in view of the results obtained in media containing different electrolytes. As shown in Fig. 3, when A23187 is added to PMNL suspended in KRT, a slight stimulation of respiration is observed. This activation is very likely due to calcium bound to leukocytes or present as a contaminant in the reagents used. In fact, if Ca^{2+} is added to the cell suspension before A23187, a much greater increase in the rate of oxygen uptake is obtained. A similar potentiation of the effect of the ionophore is also given by Mg^{2+} , which, on a concentration basis, is less active, however, than Ca^{2+} . This difference might be explained as the result of either the steeper inward gradient of Ca^{2+} concentration, or a higher sensitivity of the cytoplasmic target to Ca^{2+} than Mg^{2+} . A third possible explanation would be that high concentrations of magnesium ions displace Ca^{2+} from cell membrane binding sites, thereby making it available for complexing and transport by A23187.

Table 1 Effect of ionophores on ^{14}C -glucose oxidation by PMNL

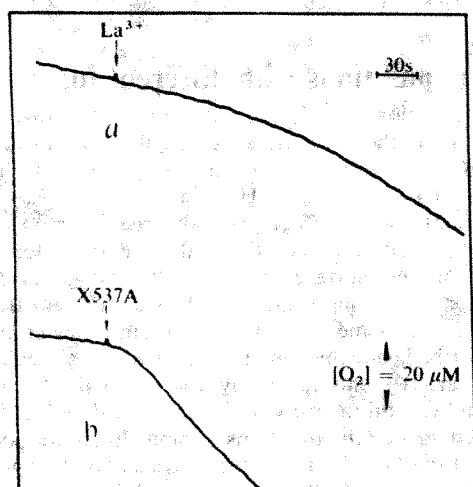
	Rest (DMSO)	X537A	A23187	Valinomycin
1- ^{14}C -glucose	16,075	61,150	33,475	30,975
6- ^{14}C -glucose	115	110	90	90

Data are expressed as $^{14}\text{CO}_2$ c.p.m. per μCi ^{14}C -glucose per 5 min per 1×10^7 cells. DMSO, 0.25%; ionophores, 20 μM . For experimental details see Fig. 2.

The activity of the divalent cations might be linked to an exchange with alkali ions. A23187 catalyses a K^+ release from erythrocytes¹⁵ and the parotid gland¹⁶, which is greatly stimulated by calcium ions. A similar migration of cations might also take place in leukocytes. Indeed, valinomycin and nigericin, which equilibrate K^+ concentrations across various membranes, cause a moderate metabolic stimulation of leukocytes. The activity of valinomycin cannot be amplified by an uncoupler such as 2,4-dinitrophenol, which enhances K^+ efflux from erythrocytes¹⁴, or by inhibitors of ATPase such as oligomycin or ouabain. Further, there is no synergy between valinomycin and A23187. Thus, while a divalent cation¹⁷ is an essential element in eliciting the metabolic response, the question whether or not K^+ participates in the process still remains open. It is important to emphasise, however, that X537A, the only ionophore among those used which is capable of carrying both alkali and alkali earth ions, provides the most efficient stimulus. Further, La^{3+} , which is also a good stimulant, has been shown both to deplete Ehrlich ascites tumour cells of K^+ (ref. 12) and displace Ca^{2+} from various biological membranes¹⁷.

Changes in the intracellular concentration of calcium ions seem to control the activity of various secretory cells. For example, A23187 and X537A cause a release of histamine from mast cells¹⁸, by promoting Ca^{2+} influx and extrusion of secretory granules¹⁹. Woodin has shown that the exocytosis of granule proteins from leukocytes treated with leukocytidin is accompanied by loss of potassium and accumulation of calcium ions²⁰. It would be expected that at least some of the agents we have used are able to promote

Fig. 1 Stimulation of the rate of respiration of guinea pig polymorphonuclear leukocytes by La^{3+} or X537A. 2×10^7 cells were suspended in, a, 2 ml calcium-free Krebs-Ringer Tris buffer (KRT-Mg) or, b, 2 ml calcium-free Krebs-Ringer phosphate buffer (KRP-Mg), containing 0.2 mM glucose (37°C). The oxygen uptake was continuously recorded with a Clark-type electrode. LaCl_3 , 0.3 mM; X537A, 20 μM .



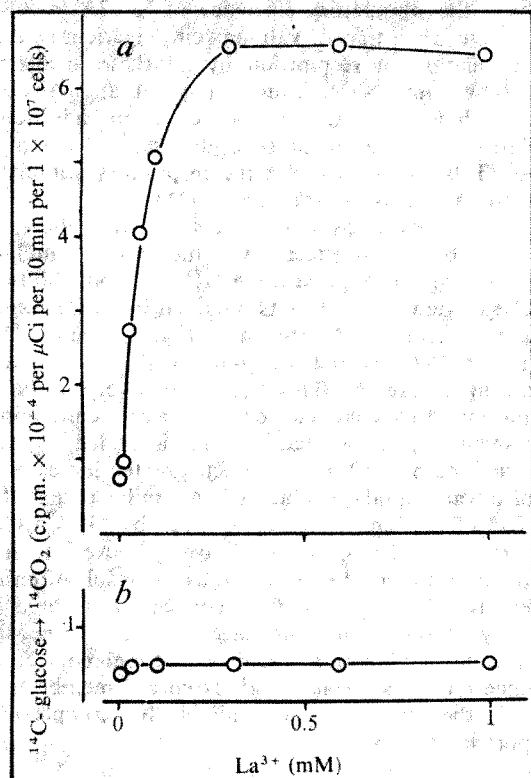
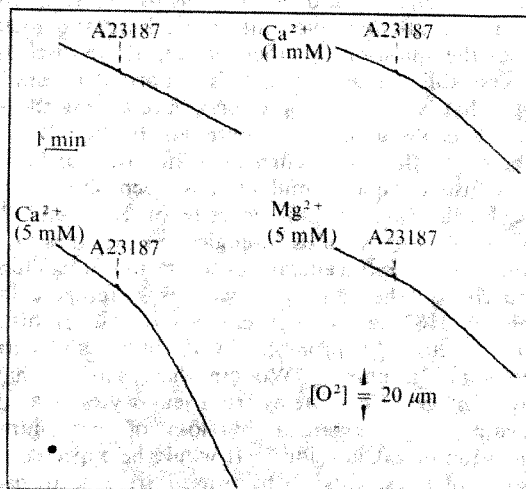


Fig. 2 Effect of lanthanum ions on the rate of ^{14}C -glucose oxidation by guinea pig polymorphonuclear leukocytes. 1×10^7 cells were incubated at 37°C in 2 ml of KRT-Mg, containing 0.2 mM glucose, in rubber-capped flasks. Different amounts of LaCl_3 were then added, followed by, a, $0.4 \mu\text{Ci}$ of ^{14}C -glucose or, b, $4 \mu\text{Ci}$ of ^{14}C -glucose. After 10 min, the reaction was stopped with 1 N H_2SO_4 ; $^{14}\text{CO}_2$ was collected in 20% KOH, present in the flask centre well, and counted by scintillation.

a release of lysosomal enzymes from PMNL. Preliminary experiments have shown that X537A causes a moderate exocytosis of β -glucuronidase from PMNL. The appearance of the lysosomal enzyme in the medium is associated, however, with a parallel leakage of cytoplasmic enzymes such as lactate dehydrogenase and glucose-6-phosphate dehydrogenase. As reported by Goldstein *et al.*²¹, A23187 exhibits a very slight potentiation of the release of β -glucuronidase from human PMNL in the presence of Ca^{2+} . Thus, the relationship between the stimulation of the PMNL oxidative metabolism by ionophores and the selective release of lysosomal proteins remains to be clarified.

Fig. 3 Effect of added Ca^{2+} and Mg^{2+} on stimulation of guinea pig leukocytes by A23187. 2×10^7 cells suspended in 2 ml of KRT, containing 0.2 mM glucose. A23187, $10 \mu\text{M}$.



In conclusion, all the results presented here may be used to build a model of metabolic excitation of leukocytes. It is tempting to hypothesise a single, unique mechanism responsible for all the effects evoked in PMNL either by phagocytosable matter or by other surface perturbing agents (anti-PMNL antibodies, phospholipase C, detergents, fatty acids, con A and so on)². The trigger mechanism would consist, in fact, in a rapid shift of divalent cations from the cell environment and/or from the plasma membrane binding sites towards special zones of the cytoplasm. Calcium ions are likely candidates for this task. Other ion species, possibly K^+ , would be released in the opposite direction at the same time. According to this picture the leukocyte excitation would follow a mechanism rather common to other excitable cells.

Admittedly, this hypothesis requires further experimentation. Measures of actual fluxes of ions, ion gradients and dependence of the metabolic effects on these parameters are now under way in our laboratory.

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T-cell populations with different functions

VARIOUS functions have been ascribed to thymus-derived (T) cells, including the ability to synergise with B cells in antibody responses¹ and with T cells in the generation of effector functions (refs 2-4 and H. Cantor and E. Simpson, unpublished), cytotoxic activity⁵, and regulator or suppressor activity^{6,7}. T-cell subpopulations, defined by criteria such as quantity of cell surface 0 antigen, homing characteristics (H. Cantor and E. Simpson, unpublished) and sensitivity to the effects of adult thymectomy and anti-thymocyte serum^{2,8}, have been described, but the functions of given subpopulations are still unclear. It seems likely, by analogy with B-cell differentiation and commitment to antibody class, that T cells become committed to certain functions during differentiation. In the absence of markers for individual T cells and their products or functions, analysis of cell populations and their activities provide

Table 1 CBA/J spleen cells sensitised to DBA/2 antigens: development of cells with helper, cytotoxic and nonspecific suppressor activity

CBA/J* spleen cell transferred	Cytotoxic activity† against:		Anti-DNP antibody response of recipients boosted with (ABC × 10 ⁻⁸ M)	
	P815	EL-4	DNP-P815§	DNP-EL-4§
0	—	—	0.49 (0.37–0.64)	—
50 × 10 ⁶ normal	0	0	0.32 (0.23–0.45)	0.07 (0.04–0.12)
50 × 10 ⁶ grafted with DBA/2 skin, day 28	0	0	19.9 (14.1–25.8)	—
50 × 10 ⁶ grafted with DBA/2 skin, day 13	0	0	24.3 (15.4–38.3)	0.14 (0.09–0.22)
50 × 10 ⁶ cultured with irradiated DBA/2 spleen cells‡	19.90 ± 0.49	5.36 ± 0.17	0.14 (0.09–0.21)	—
50 × 10 ⁶ grafted day 13 plus 15 × 10 ⁶ cultured	—	—	2.35 (2.22–2.48)	—
50 × 10 ⁶ OVA primed			DNP _s -OVA 84.8 (68.7–105)	
50 × 10 ⁶ OVA primed plus 15 × 10 ⁶ cultured			8.53 (6.43–11.3)	

* CBA/J spleen cells were sensitised to alloantigens either *in vivo* by grafting tail skin or *in vitro* by the Wunderlich and Canty¹² modification of the Mishell–Dutton culture system¹³. Some mice were primed with 100 µg alum adsorbed ovalbumin (OVA) plus pertussis. Recipients were 400 r X-irradiated CBA/J mice. All received, in addition to the helper cells shown in the first column, 40 × 10⁶ spleen cells from CBA/J mice primed with 100 µg alum-adsorbed DNP-KLH plus pertussis 4 weeks previously and treated with 0.3 ml rabbit anti-mouse thymocyte serum (ATS) intraperitoneally 48 h before transfer as a source of B-cell precursors. Boosting was with 2 × 10⁷ DNP-P815 or DNP-EL-4, or with 5 µg DNP-OVA given intraperitoneally. Mice were bled 10 d later and anti-DNP antibody was measured by a modified Farr technique¹⁴. Geometric mean antigen binding capacity (ABC) for groups of five mice was determined; the value in parentheses is the range enclosed by one standard error.

† Cytotoxicity = % specific lysis

$$\frac{^{51}\text{Cr released by sensitised cells} - ^{51}\text{Cr released by non-sensitised cells}}{^{51}\text{Cr released from } 4 \times \text{frozen and thawed targets}} \times 100$$

Titration of cytotoxic activity were performed according to the method of Canty and Wunderlich¹⁵ using a fixed number (5 × 10⁵) of ⁵¹Cr-labelled target cells and different numbers of attacking cells, usually 4 × 10⁵, 2 × 10⁵, 1 × 10⁵ and 0.5 × 10⁵ in 1-ml volumes using four replicates for each attacker: target cell ratio, that is attacker:target cell ratios of 8:1, 4:1, 2:1 and 1:1. Target cells were from the ascites tumours EL-4 (H-2^b) and P815 (H-2^d). Under the conditions used, cytotoxic activity was directly proportional to the attacker:target cell ratio. In this table, the figures for cytotoxic activity shown are for an attacker:target cell ratio of 8:1. Background spontaneous ⁵¹Cr release for P815 was 6.75% and for EL-4 was 4.35% (4 h assay).

‡ *In vitro* sensitisation: 20 × 10⁶ CBA/J spleen cells were cultured with 1 × 10⁶ 2500 r X-irradiated DBA/2N spleen cells in 1 ml volumes and collected after 5 d. Numbers of cells used in transfer refers to number of viable cells transferred.

§ P815 and EL-4 tumour cells were reacted with 1-fluoro-2,4-dinitrobenzene in isotonic carbonate-bicarbonate buffered saline, pH 9.3, at room temperature for 15 min and washed thoroughly to yield DNP-P815 and DNP-EL-4.

an approach to this question. Experiments of this type have dissociated helper and cytotoxic activity^{9,10}, and the 'unmasking' of nonspecific suppressor T cells was reported to follow the culture of helper cells with specific antigen¹¹. We have now dissociated helper activity and cytotoxic activity directed towards a given alloantigen following either *in vitro* or *in vivo* immunisation. We also found that nonspecific suppressor T cells arise during *in vitro* culture.

To investigate whether cytotoxic T-cell activity and helper T-cell activity specific for the same alloantigens occurred in the same population of sensitised T cells, CBA/J mice were sensitised to DBA/2 and their spleen cells were assayed simultaneously for cytotoxic and helper activity. In the experiment illustrated in Table 1, spleen cells from CBA/J mice immunised *in vivo* by skin grafting 13 or 28 d previously had no cytotoxic activity but did demonstrate substantial helper activity (lines 3 and 4 compared with lines 1 and 2). In other experiments, weak, variable cytotoxic activity was observed in spleen cells of skin grafted mice; this activity did not correlate with helper activity. *In vitro* sensitised spleen cells, however, had considerable cytotoxic activity (the small amount of activity against EL-4 was probably the result of H-2 determinants shared by H-2^b and H-2^d, but not H-2^k mice); such cells had no helper activity (line 5). To test for suppressor activity, small numbers of *in vitro* sensitised cells were mixed with active helper spleen cells from mice immunised *in vivo* before transfer. With 15 × 10⁶ *in vitro* sensitised cells the anti-DNP antibody response was 10% of the expected value (line 4 compared with line 6). The suppressor activity was apparently nonspecific, for 15 × 10⁶ *in vitro* sensitised cells had the same effect on the anti-DNP antibody response to DNP_s-OVA in recipients of OVA-primed helper cells (lines 7 and 8).

Further experiments to test the specificity of helper and cytotoxic cell activity and of the suppressive effect are shown in Table 2. In this experiment, spleen cells from CBA/J mice immunised *in vivo* with either DBA/2 or C57BL/6 antigens were transferred to irradiated recipients together with DNP-primed spleen cells and the appropriate antigen. *In vivo* immunised cells had helper activity specific for the priming alloantigen but

Table 2 Generation of suppressor cells in the absence of killer cells

CBA/J spleen cells*	Cytotoxic activity† against:		Anti-DNP antibody response of recipients boosted with (ABC × 10 ⁻⁸ M)	
	P815	EL-4	DNP-P815	DNP-EL-4
50 × 10 ⁶ normal	0	0	0.26 (0.18–0.36)	0.05 (0.03–0.10)
50 × 10 ⁶ grafted with DBA/2 skin, day 15	0	0	3.8 (3.0–4.8)	0.32 (0.20–0.51)
50 × 10 ⁶ grafted with C57BL/6 skin, day 15	0	0.60 ± 0.16	0.46 (0.31–0.67)	1.48 (0.73–3.00)
50 × 10 ⁶ grafted plus 20 × 10 ⁶ cultured with irradiated DBA/2 spleen cells‡	3.00 ± 0.14	0.72 ± 0.21	1.31§ (0.87–1.97)	0.64 (0.38–1.06)
50 × 10 ⁶ grafted plus 20 × 10 ⁶ cultured with irradiated C57BL/6 spleen cells‡	0	37.6 ± 0.19	1.77 (1.32–2.38)	0.51 (0.28–0.94)
50 × 10 ⁶ grafted plus 20 × 10 ⁶ cultured with irradiated CBA/J spleen cells‡	0	0	0.94§ (0.70–1.26)	0.18§ (0.11–0.27)

* DNP-B cell precursor donors treated with ATS as in Table 1.

† Cytotoxic activity, as in Table 1. Figures shown are for attacker:target cell ratios of 8:1. P815 background ⁵¹Cr release was 7.07%, EL-4, 5.87% (4-h assay).

‡ Cytotoxic data for these groups is for the cultured cells alone. After transfer of spleen cells from grafted donors, recipients were boosted with DNP on the homologous tumour cell, that is DNP-P815 for recipients of DBA/2 skin grafted donor spleen cells, DNP-EL-4 for recipients of C57BL/6 skin grafted donor spleen cells.

§ Suppression of anti-DNP response significant at *P* < 0.05 by two-tailed Student's *t* test.

Table 3 Effect of anti- θ serum and complement on cytotoxic and suppressor cells

CBA spleen* helper cells transferred	CBA cells tested for cytotoxic and suppressor activity	Cytotoxic activity† against P815	Anti-DNP antibody response of recipients boosted with DNP-OVA (ABC $\times 10^{-8}$ M)
50 $\times 10^6$ normal	—	—	0.1
50 $\times 10^6$ OVA primed	—	—	21.5
			(18.4–25.3)
50 $\times 10^6$ OVA primed	15 $\times 10^6$ normal spleen	0	32.8
			(25.5–42.1)
50 $\times 10^6$ OVA primed	15 $\times 10^6$ cultured with irradiated DBA/2 spleen cells	25.6 \pm 0.4	7.4
			(5.9–9.3)
50 $\times 10^6$ OVA primed	15 $\times 10^6$ cultured with irradiated DBA/2 and treated with anti- θ and C'‡ following collection at day 5	7.2 \pm 0.3	18.5
			(16.6–20.7)

*Cell transfers and cultures as in previous Tables.

†Cytotoxic activity as in Tables 1 and 2. Figures given are for attacker: target cell ratio of 4:1; background ^{51}Cr release for P815 was 7.97% (4-h assay).

‡Anti- θ serum; conventional AKR anti- θ C3H used at 1 ml of a 1:4 dilution per 10^6 cells; C', guinea pig complement.

little or no cytotoxic activity (lines 2 and 3). When *in vitro* sensitised cells which had specific cytotoxic activity directed against the sensitising antigen were mixed with the *in vivo* primed helper cells before transfer, all anti-DNP antibody responses decreased (lines 4 and 5), and even greater suppressive activity was given by CBA/J spleen cells held in culture for 5 d with irradiated syngeneic cells (line 6). The suppressor activity was therefore essentially nonspecific both in induction and expression.

Finally, Table 3 shows that suppressor cells in the cultured spleen cell population are T cells. CBA/J spleen cells sensitised *in vitro* to DBA/2 antigens and showing cytotoxic activity against H-2^d target cells were tested as nonspecific suppressors *in vivo* by mixing them with DNP-KLH-primed and OVA-primed CBA/J spleen cells. The anti-DNP antibody response of the recipients to DNP-OVA was suppressed by the cultured cells (line 4 compared with lines 2 and 3) but this effect was abrogated by treatment of the cultured cells with anti- θ plus complement, which also substantially reduced their cytotoxic activity. This strongly suggests that the suppressive effect is a T-cell function.

Helper activity and cytotoxic activity are both T-cell functions and demonstrate specificity for antigen. The activities are dissociated in these experiments since immunisation regimes which favour the production of one apparently induce little of the other. The suppressor activity present in those populations with high levels of cytotoxicity could, however, mask the activity of helper cells induced in the same populations. While helper and cytotoxic activities are not manifest in the same populations and are probably functions of different T-cell subpopulations, the present evidence is not decisive. Nor do these data bear on the question of whether the cells with these effector activities are derived from the same or different precursor cells. Data obtained with anti-Ly antisera before and after sensitisation suggest, however, that helper cells and cytotoxic cells occur in different populations and are derived from different precursors (P. Beverly and H. Cantor, personal communications).

The suppressor activity we have described is also a T-cell function but differs from helper and cytotoxic activity in being nonspecific both in induction and expression. The suppressor cell generated by 4–5 d of culture under Mishell–Dutton conditions is interesting for several reasons. While Kontiainen and Feldmann¹⁶ have reported the generation of specific T helper cells in Marbrook cultures, helper cell generation in Mishell–Dutton cultures has not been observed. This failure may well be due to masking of helper cells by suppressor cells. Indeed, Kontiainen and Feldmann (personal communication) found that helper activity was optimal at 3–4 d of culture and declined thereafter, perhaps due to generation of suppressor cells. Furthermore, *in vitro* primary antibody responses tend to 'turn off' after 5–6 d even when the medium is changed regularly. This phenomenon may well result from supervening suppressor activity of the type we describe. Direct evidence that this

may be occurring has recently been provided by experiments in which the *in vitro* anti-DNP response to DNP–Ficoll, a T-independent antigen¹⁷, was prolonged solely by pretreatment of the cultured cells with anti- θ and C' (D. Mosier, personal communication). The suppressor cell, described by Pierce¹⁸ and Dutton¹⁹, that arises in spleen cells cultured for 2 d with concanavalin A may be the same type of cell; the concanavalin A might serve primarily to accelerate its activation. The stimulus for the generation of the nonspecific suppressor T cells observed in our experiments is not known, as alloantigens are not required. The foetal calf sera used in these experiments, however, while from several different batches, all contained those unknown features which make for good *in vitro* immune responses (R. I. Mishell, personal communication). Such materials have mitogenic potential and this may account for the generation of suppressive activity. In parallel experiments the T-cell nature and lack of specificity of this suppressor cell have been confirmed, using an *in vitro* assay for its activity. Furthermore, it has been shown to suppress the *in vitro* antibody response to a T-independent antigen, suggesting that it may act directly upon B cells (P. C. Marrack and J. W. Kappler, unpublished).

In conclusion, T cells with three distinct activities have been demonstrated and dissociated: antigen specific helper T cells, antigen specific cytotoxic T effector cells and nonspecific T suppressor cells. Our data do not show whether these populations come from a common precursor nor, if so, at what stage they differentiated into distinct sublines of cells.

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Prolonged survival of muscle allografts in adult rats

ALLOGRAFTS from minced muscle tissue and free muscle grafts are retained, as are other types of allografts, if immunological tolerance is induced in the recipients by neonatal injection of antigen¹, but are rejected in untreated animals^{1,2}. Recovery of contractile and histochemical properties of the regenerating muscle fibres in such free muscle allografts in tolerant rats has been observed. This study was designed to determine the fate of free muscle grafts transferred to animals treated in adult life.

Rats of the AVN strain were immunised at the age of 2.5 months with antigen prepared from rats of the Lewis (Lew) strain, differing from the AVN recipients at the strong (H-1) and weak (non-H-1) histocompatibility loci. When the hydrocortisone injections were stopped, free grafts of the whole extensor digitorum longus (EDL) muscle were transplanted into the bed of the previously removed EDL muscle of the recipient. The tendon of the muscle was sutured to that of the removed muscle. The grafted fast EDL muscle undergoes a process of degeneration succeeded by regeneration during which it changes from the originally slow to a fast contracting muscle, and histochemically from a homogeneous to a mixed muscle fibre pattern. A detailed study of the changes in contractile and histochemical properties of such free grafts has been reported⁴. The recovery of grafted muscle may be complete with respect to twitch characteristics⁵ but rather incomplete with respect to weight and tetanic tension output and morphological changes⁴⁻⁷.

One group of AVN rats received transplanted muscles from Lew donors (H-1 and non-H-1 difference) and a second group from the congenic strain Lew.1A donors, differing from the AVN recipients only at the weak (non-H-1) histocompatibility locus⁸. Thirty days after transplantation and combined treatment, the EDL muscles were removed and their contractile properties studied *in vitro*. The muscles were set up in a chamber with platinum electrodes for massive stimulation⁹ at a temperature of 37°C and the contraction properties recorded using an automatic analyser¹⁰. Twitch tension, latency period,

Table 1 Contractile properties of free muscle allografts in treated animals

Type of graft	TT (g)	LP (ms)	CT (ms)	HRT (ms)	Tet.T. (g)	Weight of muscle (mg)
Lew.1A (n = 4)	2.70 ±0.63	2.95 ±0.22	17.95 ±2.01	22.50 ±2.01	10.70 ±2.86	47.05 ±9.24
Lew (n = 4)	1.46 ±0.37	2.70 ±0.30	17.38 ±1.93	22.60 ±2.93	6.03 ±2.11	46.50 ±15.05
Lew.1A + Lew	2.08 ±0.41	2.82 ±0.18	17.66 ±1.29	22.55 ±1.82	8.36 ±1.87	47.00 ±8.18

Treatment comprised immunisation of the recipients with donor antigen together with injections of hydrocortisone. Lyophilised spleen cells in saline were used as antigen and injected in fifteen doses five times per week, the first three doses being 3, 2 and 1 mg, respectively, and the subsequent doses, 0.8 mg. Hydrocortisone (Spofa, Prague) was injected in eight doses of 6.25 mg, each dose in 0.5 ml of distilled water. The first three injections were given on consecutive days and the subsequent five injections on alternate days³. Free allograft of the extensor digitorum longus muscle of weak (Lew.1A) or strong (Lew) antigenic difference transplanted into treated (hydrocortisone and injection of lyophilised spleen cells) rats (AVN). Data are given as mean ± s.e. Third row indicates results of both groups. TT, twitch tension; LP, latency period; CT, contraction time (time to peak); HRT, half relaxation time; Tet.T., maximal tetanic tension output.

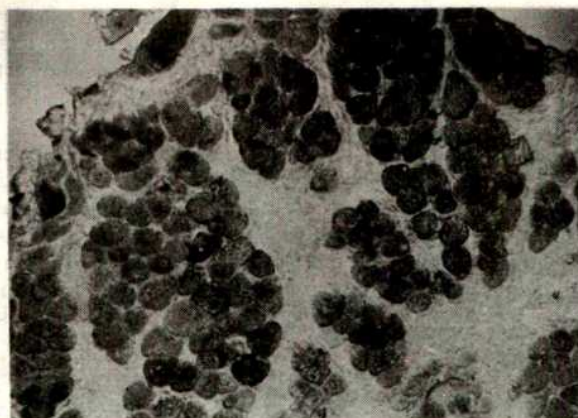


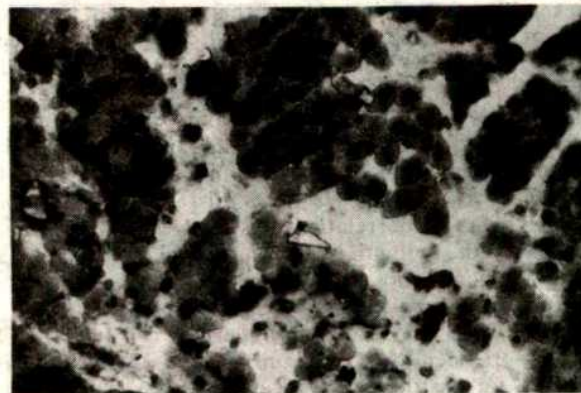
Fig. 1 Cross section through EDL muscle allograft (Lew.1A) in treated animal (AVN) 30 d after transplantation, stained for succinic dehydrogenase activity.

contraction time (time to peak), half relaxation time and maximal tetanic tension output were recorded. The muscle regenerates were removed whole and frozen quickly in CO₂; transverse sections were cut using a cryostat at 20°C and stained for succinic dehydrogenase activity, adenosine triphosphatase activity at pH 9.5 and phosphorylase activity. In some cases, routine histological observations after formalin fixation, paraffin embedding and staining with haematoxylin and eosin, were also made.

Table 1 shows the results obtained 30 days after grafting. Regeneration and concomitant recovery of contraction properties of both types of grafted muscles were observed. There was no clear difference, however, between the two groups (n = 4 in each group), that is between those with the weak antigenic difference (non-H-1) and those with the strong antigenic difference (H-1). The pooled data of both groups (n = 8) do not therefore differ from those of the individual groups. Recovery of contractile properties is somewhat less effective than that in autografts or allografts in tolerant rats¹, the contraction times being slower and the maximal tetanic tension output being slightly smaller.

Figures 1 and 2 show the regenerating muscle fibres in cross section, stained for succinic dehydrogenase (Fig. 1) and adenosine triphosphatase (Fig. 2) activity. Untreated allografts are completely rejected (six out of six), the muscles being replaced by connective tissues and fat as described previously^{1,2}. Thus, pretreatment has a considerable effect on muscle allograft survival, although absolute survival time was not investigated. In preliminary experiments, to elucidate the mechanism of allograft survival, the serum collected from the treated animals, 45 days after muscle transplantation was transferred

Fig. 2 Cross section through EDL muscle allograft (Lew.1A) in treated animal (AVN) 30 d after transplantation, stained for adenosine triphosphatase activity.



in amounts of 4 ml per animal. The serum had no effect on allograft survival in the animals with the strong antigenic difference, so far tested. Allograft survival was, however, observed in some cases of animals with weak antigenic differences.

Recently, a case of a successful free autograft of skeletal muscle in man has been described¹¹. The possibility of muscle grafts in tolerant rats¹ and of inducing immunological enhancement of muscle allografts by the treatment described suggests the possibility of muscle grafting in man. Immunological enhancement of muscle grafts by the passive administration of specific antisera will be the subject of further studies.

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Repair of potentially lethal radiation damage in mammalian cells is associated with enhancement of malignant transformation

THERE have been several attempts to define conditions necessary for the fixation of the transformed state in mammalian cells *in vitro*. The relationship between fixation and proliferation after treatment has been studied in cell cultures exposed to methylcholanthrene¹, X irradiation^{2,3} and SV40 virus⁴. Cells were exposed to the carcinogen while in the density-inhibited plateau phase of growth, and subsequently subcultured at low cell density so that they could resume cell division. Transformation frequency was always reduced by delaying subculture for 24 h or longer, suggesting that cells must divide soon after exposure to a carcinogen in order to fix the transformed state. No data were reported for subculture less than 24 h after treatment.

Recent evidence, however, has shown that important repair processes, reflected by marked enhancement in survival when subculture is delayed after irradiation, may occur in density-inhibited cells during the first few hours after treatment^{5,6}. We have now studied the dynamics of transformation during this interval; changes in survival and transformation frequency were compared. We hope this approach may offer insight into the interrelationships between the repair of lesions with the potential of being lethal to the cell, and the development or loss of the potential of a cell to become transformed.

We used a C3H mouse embryo-derived cell line (10T-1/2 clone 8) provided by C. Reznikoff and C. Heidelberger (McArdle Laboratory for Cancer Research, University of

Wisconsin). This cell line has been adapted for use in quantitative transformation studies by Reznikoff *et al.*⁷ who found no evidence for spontaneous expression of murine C-type virus in these cells⁸. Stock and experimental cultures were maintained in Eagle's BME supplemented with 10% heat inactivated foetal calf serum (both obtained from Grand Island Biological Co). Stock and experimental cultures were maintained as outlined by Reznikoff *et al.*^{7,8}, except that cultures were kept in large glass stock bottles and the medium was supplemented with penicillin (50 U l⁻¹). Plateau phase experiments were performed with cells held as confluent monolayers for at least 24 h. The medium was changed 24 h before irradiation of the cells.

Transformation was scored as outlined before^{7,9}. The tumorigenicity of radiation-transformed foci was established by reinjection of cloned foci into syngeneic mice (Table 1). All tumours grew to 2–4 cm in diameter, when the mice either died or were killed. Type I foci, or dense regions of cells seen on experimental plates, were not scored as transformed and were not tumorigenic. Type II and III foci were tumorigenic and were scored as transformants. No spontaneous transformation was seen on untreated control plates. The morphology and corresponding tumorigenicity data found with radiation-transformed foci are consistent with those of Reznikoff *et al.*⁷ for methylcholanthrene.

Figure 1 shows results of experiments in which cells were irradiated in the density-inhibited plateau phase, and subse-

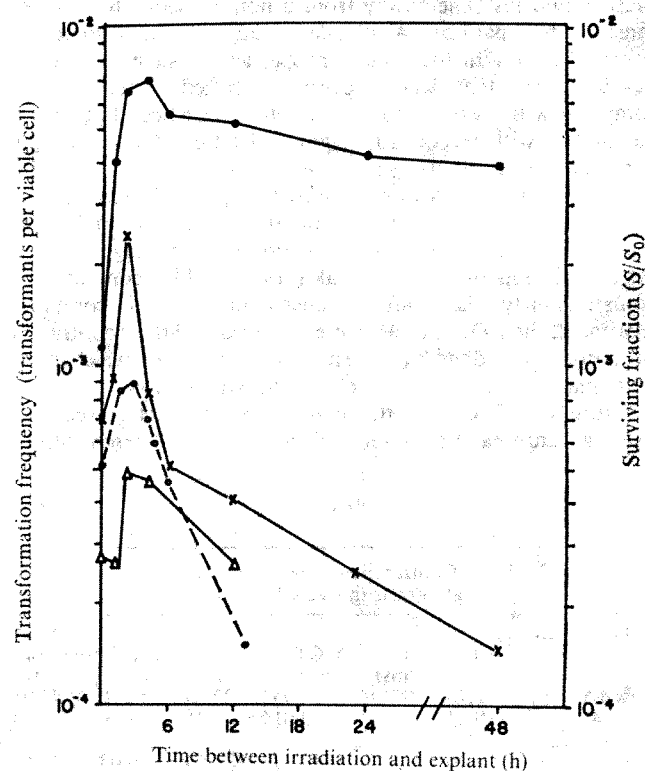


Fig. 1 Survival and transformation frequency in 10T-1/2 C3H mouse embryo cells irradiated in the density-inhibited plateau phase of growth and subcultured at various times thereafter. ●, Surviving fraction after 1,200 rad; △, transformation frequency after 200 rad; ○, 300 rad; ×, 400 rad. The confluent monolayers were trypsinised at various times (0–48 h) after irradiation, and subcultured at a lower cell density to assay for transformation and survival. Irradiation was carried out with a Phillips MG100 unit (100 kV, 10 mA) with 0.795 mm aluminium filtration and yielding a dose rate of 83.5 rad min⁻¹. For the transformation assay, cells were subcultured at a density such that approximately 200–500 viable cells per 100 mm Petri dish resulted, regardless of treatment. The medium was subsequently changed twice weekly until confluence was attained, then weekly until termination of the experiment 6 weeks after subculture. Dishes were then washed with normal saline, fixed with Bouin's solution, rinsed in 95% ethanol and stained with trypan blue. Lower density plates were also seeded at each point to assay for cell survival (plating efficiency) by standard techniques.

quently held in plateau phase for various times before subculture to assay for either survival or transformation. The upper curve, which represents cell survival, shows that survival was enhanced markedly when cells were held in plateau growth for 2–4 h after irradiation. The lower three curves represent the transformation frequency seen in similar experiments following 200, 300 and 400 rad irradiation. Transformation frequency was enhanced when cells were kept in plateau phase growth for 2–4 h after irradiation. The curves for survival and transformation were remarkably parallel for the earlier time intervals. With longer periods in plateau phase growth, however, a decline in the transformation frequency per surviving cell was consistently observed.

As Fig. 1 shows, there was an apparent increase in the amplitude of the transformation-enhancement with increasing radiation dose. A similar result has been described for the enhancement of survival, where the effect is seen as a decrease in the slope of the radiation survival curve⁶. A dose of 1,200 rad was thus chosen for the survival experiment, as the greater effect at higher doses allowed the kinetics of repair with time to be more easily studied. For transformation experiments, 200–400 rad were used as doses in this range yield maximum transformation per surviving cell⁹. The observed enhancement of survival would be fairly small following these lower doses.

These results demonstrate a high degree of correlation between the repair interval leading to both maximum survival and transformation following X irradiation. This observation sheds a somewhat different light on the relationship between delayed subculture and transformation in density-inhibited plateau phase cells as described before^{1–4}. A comparison of the data for subculture at 24–48 h after irradiation with those for 0 time shows a continual decline

a molecular level by other investigators^{10,11}. They report a peak in transformation frequency after treatment of synchronised cells with a radiomimetic alkylating agent at a time in the cell cycle when DNA strand breaks were being repaired at a maximal rate. The transformation frequency was maximal in cells treated during the 4-h interval preceding the G1/S boundary¹¹. Likewise, the repair of potentially lethal radiation damage also seems maximal in the late G1 cell population¹², the population of cells which predominates in plateau phase cultures. Taken together, these findings further support the error insertion hypothesis.

If the cell does insert the error which ultimately determines the behaviour of the cell, it may also be able to go back over its DNA and delete previously inserted errors. To be consistent with our results, this reversal of inserted errors would have to occur before the first cell division after treatment. It would thus be reflected by the decline in transformation frequency observed as the time in the density-inhibited plateau phase is lengthened. This hypothesis would also be consistent with the findings of Todaro and Green⁴ who treated cells with SV40 virus and then allowed one round of division before blocking them in a density-inhibited state. Under these conditions, no decline in the transformation frequency was observed.

Obviously, there is need for further investigation of the role of cell proliferation and molecular repair processes in oncogenic transformation; plateau cultures as a model for *in vivo* transformation may be useful. Our results indicate, however, that placing cells under conditions which enhance survival may be detrimental to the cells in terms of transformation. Furthermore, they suggest that if exposure to a carcinogen *in vivo* is associated with an appropriate proliferative response, slowly proliferating or non-actively-cycling cell populations, frequent targets of environmental carcinogens, may be particularly vulnerable to transformation.

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Table 1 Inoculation of C3H 10T-1/2 clone 8 normal and radiation transformed cells into syngeneic mice

Irradiation (rad)	Type of transformed colony	No. of cells injected per mouse	Tumour incidence (No. of tumours/No. of mice)
None	Normal cells	2–5 × 10 ⁶	0/4
600	III	2–4 × 10 ⁶	5/5
600	II	2–4 × 10 ⁶	3/4
600	I	2–10 × 10 ⁶	0/8
600	Normal (non-transformed)	2–10 × 10 ⁶	0/9

in the transformation frequency (Fig. 1). This finding is consistent with the observations with transformation induced by methylcholanthrene¹, radiation^{2,3} and SV40⁴, and with the hypothesis that if there is an interval after treatment during which a cell does not divide, it will repair the lesions or errors which would eventually lead to malignant transformation. The implication of this hypothesis is that the errors were produced as a consequence of the direct action of the carcinogen, probably on cellular DNA. Our data for repair intervals of less than 24 h suggest two alternative hypotheses: (1) errors may be inserted during the repair of radiation-induced lesions in the DNA; or (2) some severely damaged cells may repair sufficient damage to survive, but these survivors retain an unusually high risk of transformation. To explain the results in Fig. 1 by the latter hypothesis, however, the transformation frequency per cell among this repaired population would have to be three to four times the maximum frequency ever observed in irradiated cells not allowed to repair. The insertion of new errors during repair would thus seem a more reasonable explanation for the findings.

The question of whether transformation involves direct induction of the critical lesion, or whether the error is inserted in the DNA during repair, has been dealt with on

Heterotransplantation of *Theileria parva*-infected cells to athymic mice

CONGENITALLY athymic (nude) mice have been used for the heterotransplantation and maintenance of human tumours^{1–6}. In many cases aplasia of the thymus is sufficient to allow tumour growth and other immunosuppressive treatments are apparently unnecessary. Here we describe how infected cells taken from a fatal case of East Coast fever

(ECF), regularly produced tumour-like masses only in irradiated athymic mice.

ECF is a tick-borne disease caused by *Theileria parva*, an obligatory intracellular protozoan parasite which undergoes schizogony in bovine lymphoid cells and which, in infected cattle, causes lymph node hyperplasia of almost lymphosarcomatous proportions⁷. It is at present the most important cattle disease in East Africa⁸.

An outbred colony of athymic mice was established from stock supplied 9 months before experimentation, from the MRC Laboratory Animal Centre, Carshalton, UK. Mice were maintained as an isolated colony under conventional conditions; those used for experiments were 6–8 weeks old and were irradiated at 800 rad from a ⁶⁰Co source 1 h before inoculation.

A steer was experimentally infected with ECF⁹ and died 20 days later. Immediately after death one iliac lymph node and one mediastinal lymph node were removed aseptically and finely chopped in sterile Eagle's MEM with added heparin (20 IU ml⁻¹). The cell suspension was pipetted off, centrifuged at 1,000g for 10 min and the cell pellet resuspended in Eagle's MEM, supplemented with 20% foetal calf serum, L-β-asparagine and antibiotics¹⁰. Lymphoid cells (5.6 × 10⁶) were thus obtained, of which 72.5% contained *T. parva* macroschizonts and 4.0% contained microschizonts. For mouse to mouse passage, tumour-like masses were similarly treated, but after removal were trypsinised for 4 × 30 min in 0.25% trypsin at 37° C, before pelleting and resuspending in Eagle's MEM. All inocula of mice were of 5.0 × 10⁷ cells in 0.2 ml of medium, given subcutaneously behind the right shoulder.

Cells obtained from the infected steer were inoculated into each of five irradiated athymic mice, and five irradiated Swiss mice. The residual cells were set up in culture¹⁰.

Between days 8 and 9 after inoculation, subcutaneous tumour-like masses were detected at the site of inoculation in four athymic mice. These continued to grow until the mice died on days 12, 33, 38 and 40. Biopsies of the masses showed the continuous presence of morphologically-normal parasitised bovine lymphoid cells. At *post mortem* examination, extensive vascular spread of these cells was seen on examination of blood and organ impression smears. Such cells, and occasional free macroschizonts, were particularly common in the lungs and liver, but were also seen in kidney, spleen, brain and lymph nodes. The tumour-like masses were composed almost entirely of parasitised bovine lymphoid cells and microschizonts were quite common in the later stages of growth. Piroplasms invading mouse erythrocytes were occasionally seen. Karyology of cells from a mass from one of the mice showed parasites present only in bovine cells. Despite the extensive vascular spread of parasitised cells, no tumour-like masses were detected in other sites on either macroscopic or histological examination. No macroscopic evidence of residual thymus tissue was detected *post mortem* in any of the mice examined.

No tumour-like masses developed in any of the Swiss mice, but lymphoid cells put into culture grew immediately.

The mass from the athymic mouse that died on day 33 was removed and a cell suspension prepared, aliquots of which were inoculated as before into five irradiated athymic mice. All five mice developed tumour-like masses, and passage was continued in irradiated athymic mice. Six passages were carried out and parasitised bovine lymphoid cells survived continuously in the mice for 136 d. During this time 47 irradiated athymic mice were inoculated and 43 developed parasitised tumour-like masses at the site of inoculation. Four mice died before growth was detectable.

The behaviour of cells in mice was similar in all passages and no evidence of further adaptation of the parasite was observed.

At passages 4 and 5 cells were also inoculated into a total of 8 non-irradiated athymic mice; two of these mice

developed small transient tumour-like masses and one developed a large persistent mass similar to those seen in irradiated mice.

Many athymic mice died as a result of the high dose of irradiation, but in some cases death was associated with massive infiltration of parasitised cells both locally and vascularly. In these cases respiratory distress, caused by pulmonary invasion by parasitised cells, was commonly seen as a prelude to, and as a probable cause of, death. The masses, which sometimes exceeded 20% of body weight, occasionally became excoriated on the surface and avascular necrosis was common in the larger ones. There was, however, no evidence of regression or rejection.

In Swiss mice, features associated with malignant neoplasia of lymphoid cells were previously recorded, following inoculation of *T. parva*-infected lymphoid cells grown in culture^{11,12}. Our findings support the concept of neoplastic-like growth of *T. parva*-infected cells in mice and provide a further step towards developing a laboratory model for use in ECF research, for example in chemotherapy, cross immunity studies and in the isolation of field infections. The invasion of mouse erythrocytes by piroplasms of *T. parva* has not previously been reported, although infected erythrocytes have been noted in irradiated athymic mice inoculated with *T. parva*-infected bovine lymphoid cells grown in culture (unpublished data). This finding opens up the possibility of interesting tick transmission studies.

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Transplantation and preliminary characterisation of lymphocyte surface markers of Abelson virus-induced lymphomas

MOST murine lymphomas induced by commonly available murine leukaemia viruses (such as Gross passage A and Moloney leukaemia viruses) arise in the thymus, originating as thymocytes or thymus-derived (T) cells. The tumours thus usually carry the surface θ antigen², they rarely

Table 1 Transplantation of Abelson virus-induced lymphosarcomas

Primary tumour No.	Days from virus injection to diagnosis of primary tumour	No. of primary cells injected i.p. $\times 10^6$	Frequency of ascites tumours in recipients		Time for development of ascites tumours in pristane-primed mice: mean and (range)	Times for development of non-ascites tumours in control mice: mean and (range)	
			Control	Pristane primed		Needle-track	Lymphoma
1	21	5	0/3	2/3	13(13)	19	43(43)
2	22	16	0/3	3/3	14(13-15)		44(36-60)
3	24	14	0/3	2/3	10(10)		55(51-57)
4	25	35	0/4	4/4	14(10-22)		47(36-50)
5	26	26	0/4	4/4	12(11-15)	18,18	42(35-49)
6	30	1	0/3	3/3	29(19-38)		43(38-45)
7	34	2.5	0/3	3/3	14(12-17)		39(34-41)
8	35	8.9	0/3	3/3	25(22-26)		39(33-51)
9	21	3.8	0/3	3/3	25(25)		48(45-53)
10	21	2.5	1*/3	3/3	9(8-10)	11*,15	32(32)

Abelson virus was obtained from Dr L. Rabstein, passaged in BALB/cAnN mice as 10% (w/v) tumour extracts. The pools used had a plaque-forming titre of 2×10^6 MLV PFU per 0.1 ml as measured by the XC test⁷ and a tumorigenic dose 50 (TD₅₀) of 2×10^2 per 0.1 ml when injected in newborn BALB/c mice. All pools also contained the LDH virus (which is not necessary for lymphoma induction by Abelson virus). Newborn mice were injected with 0.05 ml virus intraperitoneally (i.p.). Tumours arose in lymph nodes, meninges and bone marrow 3-5 weeks after injection (column 1). For transplantation, tumour cell suspensions were injected intraperitoneally into female BALB/c mice 2-4 months old, some pretreated 7-28 d previously with 0.5 ml pristane intraperitoneally (in transplantation of plasmacytomas, the pretreatment interval does not seem to be critical for transplantation enhancement). Ten primary tumours were used as the source of malignant cells to inject 32 pretreated mice and 32 controls.

* Same tumour.

have B-cell surface markers such as the κ (light) chain. Abelson and Rabstein, however, reported a leukaemia virus that induces non-thymic lymphomas³⁻⁵, which we have now found to have surface κ chains under certain circumstances.

When we started this work, transplantation of Abelson virus-induced tumours had not been reported. We therefore pretreated the recipients of the transplants with 0.5 ml of pristane intraperitoneally, as this had been previously shown to enhance the transplantation of plasma cell tumours⁶. As Table 1 shows, pristane-priming makes a striking difference in the efficiency of transplantation and the pathology of the transplanted tumour. Of the control mice, not treated with pristane, only 1 out of 32 developed an ascites tumour while four others developed local needle-track tumours (the control mouse with ascites also had a needle-track tumour). Local needle-track tumours presumably represent cellular transplantation rather than local virus induction of tumour. Of more than a thousand mice injected by us with Abelson virus, none has ever developed a needle-track tumour. The other 27 control mice developed a generalised non-thymic lymphoma after a mean latent period of 44 d (range 32-60 d). The gross distribution and microscopic pathology of the lymphomas arising in this group of mice was identical to that which results from inoculation of the cell-free virus into adult BALB/c mice. The pathology of these mice, infected with virus as adults, differs from that of those infected with virus as newborns by its longer latent period and the rarity of cranial bulging and facial tumours. Since the transplanted tumours all actively produce virus, the late-arising tumours in the non-pristane-treated mice very likely represent fresh tumour induction by virus. The later time of onset (32-60 d) of these tumours corresponds to those previously published for virus-induced disease in adult BALB/c mice⁸.

On the other hand, 30 out of 32 of the pristane-primed recipient mice developed ascites tumours, most of them very rapidly. It should be noted that seven out of the ten primary tumours transplanted successfully as ascites tumours with mean latent periods of 9-14 d. Successful transplantation of three of the ten primary tumours took longer, with means of 25-29 d. Since we have never obtained tumours in primed mice or unprimed mice by straight virus injection in less than 20 d we conclude that, at least 7/10 primary tumours were successfully transplanted. More rigorous evidence that these tumours were indeed transplants rather than fresh virus inductions was provided by karyotyping four tumours which were transplanted into mice of the opposite sex (XX or XY karyotypes). Secondary

or later passages of tumour No. 10 and three others not listed in Table 1 were all the karyotype of the donor (primary) mouse. Thus, it seems that the pristane-primed mouse is a far superior recipient for the successful transplantation of this type of tumour.

One ascites tumour arising from the transplantation of each of the 10 primary tumours was studied for the presence of lymphocyte surface markers (Table 2). Each of the 10

Table 2 Surface markers on tumour cells

Tumour No.	K ⁺	Primary		θ	Transplant	
		EA/EAC	EA/EAC		K ⁺	EA/EAC
1	—	—	—	—	+	—
2	—	—	—	—	+	—
3	—	—	—	—	+	—
4	—	—	—	—	+	—
5	—	—	—	—	+	—
6	—	—	—	—	+	—
7	—	—	—	—	+	—
8	+	—	—	—	+	—
9	+	—	—	—	+	—
10	—	—	—	—	+	—
Others†	5/18†	0/3†	0/2†	22/23*†		

The gamma globulin fraction of a rabbit anti-mouse κ chain anti-serum (a gift of Dr R. Mage) was conjugated with fluorescein isothiocyanate according to the method of Wood *et al.*⁹. Anti- θ C₃H was prepared according to the method of Reiff and Allen¹⁰. One-tenth millilitre of a cell suspension containing 5×10^6 tumour cells was incubated at 4°C with 0.1 ml of the fluoresceinated anti- κ reagent. The cells were then washed three times and one drop of the suspension was examined under ultraviolet light with a Leitz Ortholux microscope. If no immunoglobulin was detected with the direct technique by using the fluoresceinated anti- κ reagent, the tumours were then studied for the presence of the θ determinant by an indirect technique in which the cells were first incubated with the anti- θ diluted 1:10 as a middle layer followed by washing and then by the fluoresceinated anti- κ reagent. In this manner the fluoresceinated anti- κ reagent would bind to the κ chains of the mouse anti- θ antibody present on the θ -positive cells. For the detection of the C3 receptor of the B lymphocyte and the receptor for cytophilic antibody of the monocyte by red cell rosette techniques, the following reagents were prepared: 7SEA were prepared by adding the 7S rabbit anti-sheep cell antibodies (A) to sheep erythrocytes (E). 19SEAC were prepared by adding 19S rabbit antibody to E followed by an equal volume of fresh mouse serum (as the source of complement) diluted 1:10 in Veronal-buffered saline (VBS). These reagents were used as previously described¹¹. Rosette formation with the 19 SEAC reagent indicates the rosetted cells bear the C3 receptor of B lymphocytes; rosette formation with the 7S EA would indicate the cell had the cytophilic receptor of the monocyte. Under the conditions used the 7SEA reagent does not bind to the FC receptor of B cells¹⁰.

* One negative was a needle-track tumour.

† Abelson tumours not listed in Table 1.

transplanted ascites tumours listed in Table 1 as well as seven of the primaries and several other primary and transplanted tumours (shown as others on Table 2) was tested for the presence of surface κ chain. Seven tumours (Nos 1, 2, 5, 7 and three others) were also tested for their ability to form rosettes with EA or EAC. Six κ negative tumours (Nos 2, 4, 5, 7 and two others) were also tested for the presence of θ antigen. (The method used, indirect immunofluorescence, did not permit testing for θ in the presence of surface κ chain.)

The results of these tests were that all transplanted tumours had surface κ chain; 7 out of 25 primary tumours also had surface κ chain; 0 out of 6 tumours tested had θ antigen, and 0 out of 7 tumours tested formed rosettes with EA or EAC. These latter results indicated that the cells lacked the B-lymphocyte receptors for C3 and the monocyte receptor for cytophilic immunoglobulin.

The Abelson lymphomas are non-thymic in origin and, as demonstrated here, lack θ antigen; hence, they are unlikely to be derived from T lymphocytes. The basic question then is whether these cells are B-lymphocyte tumours, that is neoplastic derivatives of immunoglobulin-producing cells. The presence of κ chain on the surface of all the transplants and some of the primaries observed in this study suggests that this is indeed the case. It does not, however, prove that this surface immunoglobulin was synthesised by these cells. Another possibility is that the surface κ chains present on the tumours represent passively acquired host antibody directed against viral or tumour antigens on the surface of the neoplastic cells. While the data presented here do not rule out the latter possibility, we have in a separate report demonstrated IgM synthesis by long term tissue-culture sublines of tumour No. 5 (Table 1) (M.D.S., E. Premkumar, M.P. and P. Singer, manuscript in preparation). In addition, tumours No. 1 and No. 8 have also been shown to synthesise IgM (E. Premkumar, *et al.*, unpublished).

The results of this study together with the previous work⁴ on plasma cell tumour induction by Abelson virus indicate that this virus preferentially induces tumours in immunoglobulin-synthesising cells. The ability to obtain B-cell lymphomas rapidly, in great numbers, and perhaps with predetermined specificities, should be of great value to the cellular immunologist.

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Local anaesthetics increase susceptibility of untransformed cells to agglutination by concanavalin A

CELL agglutination by concanavalin A (con A) is accompanied by redistribution of con A receptors on the cell surface from a random pattern to form patches¹⁻⁵. Differences in the susceptibility of untransformed and transformed cells to agglutination by con A may therefore reflect differences in the ability of receptors to move laterally within the membrane to form patches. For example, it has been suggested⁶⁻⁸ that the higher susceptibility of transformed cells to agglutination by low doses of con A might be associated with a more fluid lipid plasma membrane matrix. Enhanced fluidity of the bilayer matrix would allow greater lateral movement of receptors within the membrane and favour formation of patches of receptors after binding of con A to the cell surface. The increased susceptibility of L cell nutritional auxotrophs to agglutination by con A after enrichment of their membranes with fluid phospholipids⁹ supports this view. We report here that local anaesthetics which increase the fluidity of phospholipids in model membranes also cause a significant increase in the susceptibility of untransformed cells to agglutination by low doses of con A.

BALB/c mouse 3T3 cells, 3T3 cells transformed by SV40 (SV3T3), BHK cells and BHK cells transformed by polyoma virus (PY-BHK) were grown in Dulbecco's modified Eagle's MEM in 50 mm plastic Petri dishes (Falcon) as before¹⁰. Preparation of affinity-chromatography-purified con A, measurement of ³H-con A binding to cells and quantitation of cell agglutination have also been described previously⁴. Cells for freeze fracture studies were suspended in a solution of glycerol (30% v/v), sucrose (0.25 M) and Tris (5 mM), pH 7.2, and centrifuged; cell pellets were frozen rapidly in Freon 22 and cleaved in a Balzers BA 360 freeze-etching apparatus and shadowed with platinum as before¹¹.

Maximum agglutination of untransformed 3T3 or BHK cells by con A required significantly larger doses of con A than their virus-transformed derivatives (Table 1). Incubation of 3T3 and BHK cells with the local anaesthetic dibucaine for 60 min at 37°C however, significantly enhanced their susceptibility to agglutination by con A (Table 1). No significant change (<10%) in ³H-con A binding to dibucaine-treated cells was detected. The susceptibility of 3T3 cells to agglutination by low doses of con A (350 μ g ml⁻¹) has also been enhanced by treatment for 1 h at 37°C with the local anaesthetics tetracaine (1 \times 10⁻⁴ M) and procaine (1 \times 10⁻² M).

Some effects of local anaesthetics on membranes are believed

Table 1 Effect of dibucaine on cell agglutination by con A

Treatment	Concentration of con A (μ g ml ⁻¹) for maximum cell agglutination*			
	3T3	SV3T3	BHK	PY-BHK
Untreated control	1,400	50	850	80
Dibucaine (1 \times 10 ⁻⁴ M)†	350	50	100	50
Dibucaine (1 \times 10 ⁻⁴ M) plus 5 mM CaCl ₂ ‡	1,250	50	850	80
Dibucaine (1 \times 10 ⁻⁴ M) plus 10 mM CaCl ₂ ‡	1,400	50	850	80

* 90% of cell population agglutinated as determined by microscopic counts after incubation of cells with con A for 20 min at room temperature using methods described previously⁴.

† Cells were incubated in suspension in medium containing 1 \times 10⁻⁴ M dibucaine (K and K Laboratories, Plainview, New York) for 1 h at 37°C before testing for their con A agglutinability.

‡ Cells were incubated in suspension in medium containing 1 \times 10⁻⁴ M dibucaine supplemented with 5 or 10 mM CaCl₂ for 1 hour at 37°C before testing for their con A agglutinability.

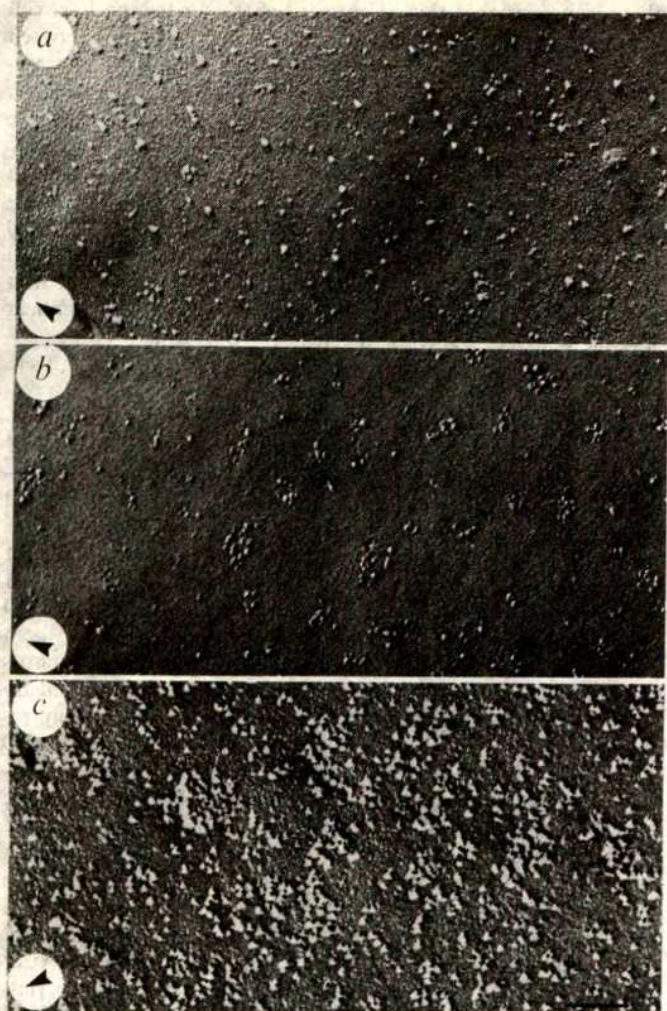


Fig. 1 Freeze-fracture electromicrographs (A face) of mouse 3T3 cells (100,000). The bar represents 0.1 μm . The arrows in the lower left corner of each micrograph indicate the direction of shadowing. *a*, Area of untreated control 3T3 cell showing predominant monogranular distribution of intramembranous particles. *b*, Area of 3T3 cell after incubation for 1 h at 37°C in medium supplemented with 1×10^{-4} M dibucaine showing increased clustering of intramembranous particles. *c*, Area of 3T3 cell after incubation for 1 h at 37°C in medium supplemented with dibucaine followed by incubation with 500 μg con A ml^{-1} for 15 min at 37°C, showing even more marked clustering of intramembranous particles than in (*b*).

to result from their ability to displace Ca^{2+} associated with the anionic groups of acidic phospholipids¹², and certain effects of these drugs can be inhibited competitively by increasing the Ca^{2+} concentration¹³. Similarly, incubation of 3T3 or BHK cells in medium supplemented with dibucaine plus additional Ca^{2+} (5 mM or 10 mM CaCl_2) eliminated the effect of dibucaine on cell agglutination by con A (Table 1).

Freeze-fracture observations of the surface of untreated 3T3 cells revealed a random and mostly single distribution of intramembranous particles (Fig. 1*a* and Table 2). After incubation with dibucaine, clusters of more than five particles were more common (Fig. 1*b* and Table 2). When similar dibucaine-treated cells were exposed to con A, more than 75% of the particles were clustered (Fig. 1*c* and Table 2). Thus, dibucaine causes a change in the structural organisation of the plasma membrane in 3T3 cells which is reflected in freeze-fractured images as redistribution of intramembranous particles, and this change is exaggerated in the presence of con A.

Electron spin resonance measurements on natural membranes¹⁵ and model membranes composed of phospholipid bilayers¹⁶ have shown that local anaesthetics increase the fluidity of phospholipids. Similarly, our data (in preparation)

from fluorescence polarisation measurements on dibucaine-treated cells—using the plasma membrane probe, diphenyl hexatriene⁷—suggests that dibucaine causes a small but significant increase in the fluidity of membrane lipids. It is not known whether this is a small change in the overall fluidity of the bulk lipid matrix or a much larger fluidity change in specific regions of the membrane. Differential scanning calorimetry and fluorescence polarisation studies on model bilayer membranes indicate that dibucaine causes a marked fluidising effect on several types of phospholipids (in preparation). At dibucaine concentrations (1×10^{-4} M) that enhance cell agglutination, however, this effect is confined to acidic phospholipids. This effect is more marked in acidic phospholipid membranes stabilised previously by Ca^{2+} , indicating that increased fluidity can also result in part from the displacement of membrane-bound Ca^{2+} by dibucaine. The same concentration of dibucaine caused no significant fluidity change in neutral phospholipids such as lecithin which constitute most of the plasma membrane lipids in mammalian cells. Thus, in intact cells low concentrations of dibucaine might induce a large fluidity change in specific membrane domains containing acidic phospholipids, without significant change in the fluidity of the bulk membrane lipid. Although such domains have not been demonstrated, experiments with mixed lipid bilayers indicate that Ca^{2+} can induce segregation of acidic phospholipids into separate domains from neutral phospholipids^{17,18}.

Table 2 Effect of dibucaine and con A on the density and distribution of intramembranous particles on freeze-fracture faces of 3T3 cell membranes

Treatment	Density of intramembranous particles (No. per μm^2)	Single	Distribution of intramembranous particles† (%)	
			Clusters < 5	Clusters > 5
Untreated control	495 ± 39	44.8	38.0	17.2
1×10^{-4} dibucaine‡	476 ± 23	21.9	41.6	36.5
1×10^{-4} dibucaine plus 500 μg ml^{-1} con A§	515 ± 35	23.5	28.7	47.8

* Mean value from counts of intramembranous particles on two or more 0.1- μm^2 surface areas of A fracture face of individual 3T3 cells at a magnification of 100,000 and counts on at least fifteen individual cells. These values are in good agreement with those reported by Scott *et al.*¹⁴.

† Percentage of total number of intramembranous particles.

‡ Cells were incubated with dibucaine for 1 h at 37°C.

§ Cells were incubated with dibucaine for 1 h at 37°C followed by addition of 500 μg ml^{-1} con A and further incubation for 15 min at 37°C.

In addition to the fluidising effect, displacement of Ca^{2+} might induce changes in the organisation of peripheral proteins on the inner face of the plasma membrane which have been suggested to act as regulatory restraints in determining the movement of receptors on the outer face of the plasma membrane¹⁹⁻²². For example, spectrin, which is involved in the trans-membrane control of lectin receptor site distribution in erythrocytes²⁰, is released from membranes by Ca^{2+} -chelating agents²³. Moreover, spectrin interacts with acidic phospholipids²⁴ at pH 7.4 only in the presence of Ca^{2+} . By competing for such Ca^{2+} -binding sites dibucaine could affect the interaction of peripheral spectrin-like proteins with membrane lipids.

The two mechanisms proposed here whereby local anaesthetics displace Ca^{2+} from acidic phospholipids and alter the function of certain peripheral proteins on the inner face of the plasma membrane could operate in parallel or in tandem. A framework of proteins on the inner membrane face exerting trans-membrane control of the topography of receptor moieties on the outer membrane face could be linked, as in the case of spectrin, to acidic membrane lipids by Ca^{2+} . This type of organisation could provide a mechanism for changes in the mechanical

properties of the plasma membrane regulated by Ca^{2+} , which would not require constant or extensive changes in the overall fluidity of the bulk lipid matrix of the membrane.

When this work was being prepared for publication, Ryan *et al.*²⁵ reported that local anaesthetics inhibited antigen-induced capping of immunoglobulin molecules on the surface of B lymphocytes. They found, however, that the formation of patches of Ig molecules, which is the process analogous to clustering of con A receptors on non-lymphoid cells, was not inhibited by local anaesthetics, but no data were given as to whether patching on lymphocytes was actually enhanced.

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Asymmetry of influenza virus membrane bilayer demonstrated with phospholipase C

In the red cell, the membrane carbohydrates¹, proteins²⁻⁹ and lipids⁹⁻¹⁶ are all unequally distributed between the two sides of the plasma membrane. The membrane proteins and carbohydrates of influenza virions are also asymmetrically distributed^{16,17} (review in ref. 18). We have now found that the phospholipids of influenza virions are asymmetrically distributed as well. Using phospholipase C (PLase C) to digest the phospholipids exposed on the outer surface we have found a predominance of phosphatidyl choline (PC) and sphingomyelin (Sph) on the outer surface and a predominance of phosphatidyl ethanolamine (PE) and phosphatidyl serine (PS) on the inner surface. Since influenza virions acquire their lipid bilayer by budding out from the membrane of their host cells, it is reason-

able to surmise that this asymmetry characterises the host cell plasma membrane as well. Thus, the consistency between our present findings and previous findings with red cell membranes⁹⁻¹⁵ suggest that an asymmetric phospholipid distribution of this type may be a general feature of plasma membrane structure.

The WSN strain of influenza A₀ virus was grown in Maden bovine kidney cells, and purified as before^{19,20}, except that a constant specific activity of ³²P-inorganic phosphate (2-5 $\mu\text{Ci ml}^{-1}$) was maintained in the medium throughout the cycle of cell growth and virus infection and multiplication. This assured that all phospholipid synthesised during this period had identical specific activity. Spikeless particles, from which the glycoproteins had been removed by treatment with bromelain, were prepared as before^{16,20}.

PLase C of two specificities was used. PLase C from *Clostridium welchii* (Sigma) was dissolved (0.5 mg ml^{-1}) in 0.05 M Tris, 0.02 M CaCl_2 , pH 7.4, and heated at 95° C for 15 min before use²¹. PLase C from *Bacillus cereus* (Sigma) was dissolved (40 U ml^{-1}) in 0.05 M Tris, 0.01 M CaCl_2 , 0.01 M MgCl_2 , pH 7.4, and heated at 56° C for 30 min before use. One volume of enzyme was added to 4 volumes of viral particles in 0.05 M Tris, pH 7.4, and the mixture was maintained at 37° C for 60 min. These conditions were chosen after trichloroacetic acid (TCA) precipitation had shown that 45-55% of the total phospholipid was hydrolysed and additional incubation caused no further phospholipid hydrolysis. The virions were then resedimented in a 5-40% potassium tartrate gradient at 35,000 r.p.m. for 90 min in a Spinco SW 50 rotor. The enzyme-treated particles, whether intact or spikeless, and their respective untreated controls sedimented similarly and seemed unchanged when negatively stained specimens were viewed in the electron microscope. The purified virions were then extracted by the method of Folch *et al.*²². The extract was separated on silica gel thin-layer plates (Eastman Chromagrams) using the solvent chloroform-methanol-acetic acid-water (25:15:4:2)^{23,24}. After development, fractions from the plates were scraped into vials and radioactivity was counted. Approximately 90% of the total ³²P of the virions was recovered in the organic phase after extraction, and recovery of counts from the plates exceeded 95% of those applied.

Table 1 Hydrolysis of phospholipids from intact and spikeless influenza virions by PLase C

Phospholipid	% Hydrolysed		<i>B. cereus</i> enzyme
	<i>Cl. welchii</i> enzyme		
	Intact	Spikeless	
Sph	51	71	0*
PC	80	84	83
PS + PI	0*	0*	37
PE	43	56	42
Total hydrolysis	43	55	46

* Assumed as basis for calculation.

In Fig. 1a and b intact particles treated with the two types of PLase C are compared with their respective untreated controls. Figure 1c shows spikeless virus particles treated with the *Cl. welchii* enzyme^{16,20}. The results are tabulated in Table 1 based on normalisation to 100% recovery (that is, no hydrolysis of PS after treatment with *Cl. welchii* enzyme^{14,25} and 100% recovery of Sph after treatment with *B. cereus* enzyme¹⁵). Values of total hydrolysis obtained in this way (Table 1) are in good agreement with the values obtained by TCA precipitation of unhydrolysed phospholipids.

The patterns of hydrolysis by the two enzymes are quite similar if differences in the inherent specificities are considered. Both enzymes hydrolysed 80-83% of the PC of the intact virion and 42-43% of the PE. *Cl. welchii* enzyme hydrolysed 51% of the Sph from the intact virion, but 71% was removed from the spikeless particle. On the other hand, only 37% of the PS was hydrolysed by the *B. cereus* enzyme. This pattern of hydrolysis does not reflect the specificity reported previously for

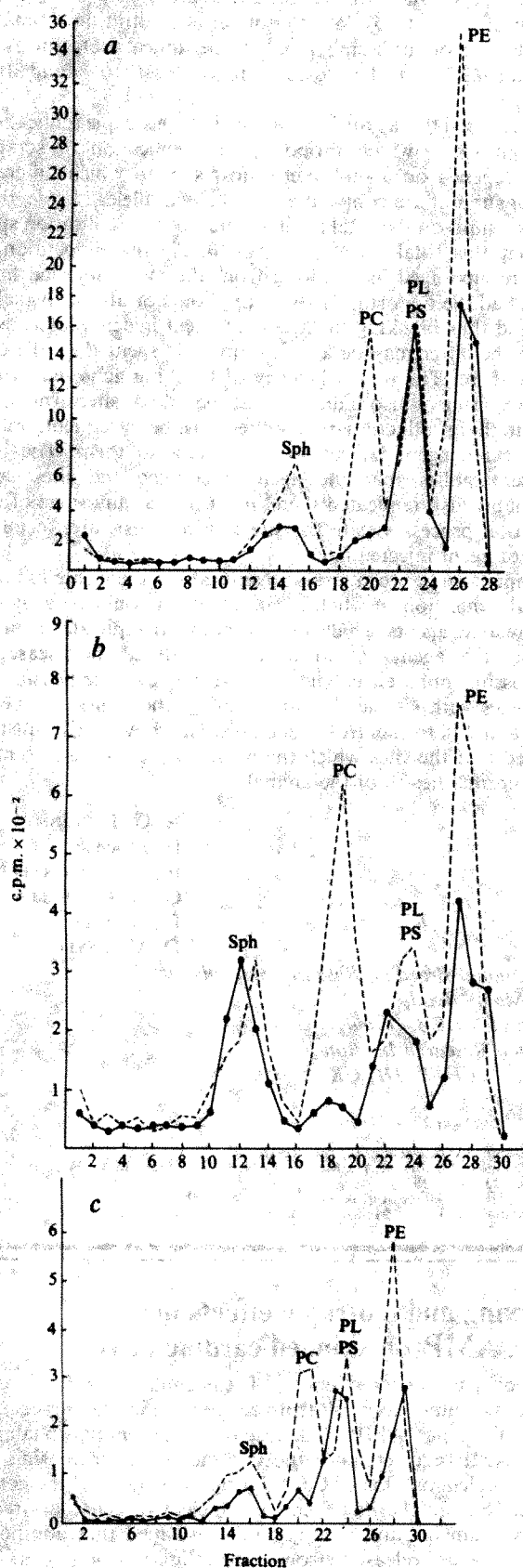


Fig. 1 Thin-layer chromatograms of phospholipid extracts of influenza virions after treatment with PLase C. Extracts from enzyme treated and control preparations were chromatographed separately, but are plotted together to facilitate comparison. —, Untreated control; —, PLase C-treated. *a*, Intact virions treated with *Cl. welchii* enzyme; *b*, intact virions treated with *B. cereus* enzyme; *c*, spikeless particles treated with *Cl. welchii* enzyme.

these two enzymes, both of which have been reported to be fully active against PE, while the *B. cereus* enzyme is also active against PS^{14,15,25}. Indeed, the PE and PS in the particles are almost completely hydrolysed by the *B. cereus* enzyme under appropriate conditions, for example after prolonged storage resulting in particle deterioration, or if divalent cations are omitted from the reaction medium. The overall similarity between results obtained with intact and spikeless particles shows that the results do not reflect the protection of particular phospholipids by the glycoprotein spikes of the intact virion.

The interpretation of these results in terms of asymmetry of the viral bilayer depends critically on the assumption that the virion is impermeable to PLase C. Influenza virions are known to be impermeable to proteases and ribonuclease, strongly suggesting that they are impermeable to other enzymes as well. Further, our results were obtained only with virions which were repurified after treatment with PLase C, thus ensuring that any disrupted particles were removed before lipid analysis. Finally, the observation that additional time of incubation, or incubation with additional enzyme, produced no additional hydrolysis suggested that a stable situation was reached in the particle in which the unhydrolysed phospholipids were unavailable to the enzyme. The penetration of the enzyme to the inner surface of the viral bilayer thus seems very unlikely.

Although our results do not permit detailed quantitation of the lipid distribution of each side of the membrane, they indicate an asymmetric distribution. PC and Sph are preferentially located on the outer surface of the viral bilayer, where they are readily accessible to externally added PLase C, while PS and PE are less accessible to the enzyme, suggesting preferential localisation on the inner surface of the bilayer. We believe that more Sph than the 50–70% hydrolysed is located on the outer surface, since Sph is known to impart resistance to digestion by *Cl. welchii* enzyme²¹, and an increase in the total amount of Sph in the viral particle results in decreased hydrolysis of this phospholipid (J. L. and K.-H. T. unpublished). On the other hand, the finding that no more than 80–85% of the PC is susceptible to hydrolysis suggests the location of the remaining 15–20% on the inner surface, since this phospholipid is readily hydrolysed by both enzymes. The results also suggest that part of the PS and PE is located on the outer surface.

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Extraneural competition between different scrapie agents leading to loss of infectivity

MANY strains of scrapie agent have been isolated which differ in their biological properties, such as incubation period and type of brain lesion¹. We have shown previously that competition occurred between different scrapie agents intracerebrally injected into mice², indicated by an increase in the incubation period of the lethal scrapie agent when a different scrapie agent had also been injected to impede its pathogenesis. We did not establish whether the increased incubation resulted from replication of the lethal agent being hindered throughout or at particular stages of pathogenesis, or whether the increased incubation period resulted from some loss of effective titre.

The pathogenesis of different scrapie agents injected into mice can only be described as relatively 'quick' or 'slow' if the recipient mouse genotype is specified in terms of the gene *sin*c (alleles *s7* and *p7*). On this basis, inbred mice can be injected with a 'slow' agent which can block, partially or completely, the pathogenesis of a 'quick' agent injected later by the same route. We have previously found that the effectiveness of blocking depends on the route of injection, the interval between injections and the particular strains and doses of agent used. We show that agent competition can be so effective in some circumstances that the 'quicker' agent, injected later, seems to take no active part in the disease and may have been entirely degraded or excreted.

RIII mice (3-4 weeks old) were injected intraperitoneally with either brain homogenate containing 22A agent, or with a similar dose of normal VM brain as controls. All mice received a second intraperitoneal injection of brain homogenate containing 22C agent, given either 100, 200 or 300 days after 22A (Table 1). The expected incubation periods for 22A and 22C given singly in these doses would be 550 days and 230 days, respectively. To calculate incubation periods when two agents are injected, we must be able to determine which agent eventually kills the mouse or, even, whether both share in this process. 22A and 22C are quite different in the intensity and distribution of brain lesions which they produce (ref. 3 and H.F., unpublished), and consequently the 'lesion profile' for each group in the competition experiment can be used to decide which origin to use for calculating incubation periods.

Table 1 Scrapie in RIIIrososines^{s7s7} mice injected intraperitoneally either with two different scrapie agents or with only one agent; the interval between injection of the two agents was varied

First injection:	Normal brain	22A
Second injection:	22C	22C
Injection interval (days)	Brain lesion profile type	Incubation period (days ± s.e.)
100	22C	237 ± 6 (7)
200	22C	232 ± 2 (7)
300	22C	231 ± 0 (5)
Injection interval (days)	Brain lesion profile type	Incubation period (days ± s.e.)
100	22A	554 ± 13 (6)
200	22A	557 ± 5 (9)
300	22A	561 ± 7 (7)

Number of mice in parenthesis. Doses: 22A agent-0.02 ml of 10⁻¹ saline homogenate from 22A-infected VM brain, containing 10⁶ VM intracerebral LD₅₀ units 0.02 ml⁻¹. 22C agent-0.02 ml supernatant (2,000g for 15 min) of 10⁻² saline homogenate from 22C-infected C57BL brain, containing 10^{3.7} C57BL intracerebral LD₅₀ units 0.02 ml⁻¹.

Table 1 shows that the 22A blocking injection was so effective that the second injection (22C) failed to infect the mice. The lesion profiles for the blocked and control groups are significantly different (method of least squares; *P* < 0.001) and the profile in the blocked animals confirms that 22A was responsible for killing the mice. If 22C does take an active part in the disease in the blocked groups, although subsidiary to 22A, we would expect variation between the results for the three sub-

groups with different intervals between injections: there was no evidence of such differences (incubation period *P* > 0.6; lesion profile *P* > 0.35). We do not yet have titration estimates in RIII mice of the number of intraperitoneal LD₅₀ units of 22A and 22C but the doses were at least 10^{1.5} and 10², respectively.

We regarded this as further support for the scrapie replication site hypothesis⁴, which proposes that replication of scrapie agents depends on a multimeric host site, to which different types of subunit are contributed by the two alleles of *sin*c, that there is a limit on the total available number of replication sites and that this total is relatively small. Agent competition is therefore envisaged as resulting from the agent injected first, having had the opportunity to occupy some or all the available sites, and thus blocking the access of agent injected later, even though the latter may be a much 'quicker' agent than the one already there. The total efficiency of blocking achieved in the above experiment also indicates that the rate of site turnover is low, and that production of new sites must be infrequent, unless the blocking agent has priority in access to them. The low turnover implies that the agent occupying the sites, and presumably also replicated there, must not, at any stage of the replication process vacate the sites, even transiently, to allow access of agent injected later.

Competition between scrapie agents able to replicate in mice, raises the question of whether site blocking could be achieved using related agents which do not seem to replicate in mice, such as the agents of kuru, Creutzfeldt-Jakob disease or transmissible mink encephalopathy. It may even be possible to block sites with simpler, non-replicating, molecules. To be of practical use in the control of such diseases, however, it must be assumed that the sites which these agents use are not essential to the normal health of the animal.

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Relaxing and inotropic effects of cyclic AMP on skinned cardiac cells

THE positive inotropic effect of catecholamines in cardiac muscle is assumed to result from an increase in the intracellular level of cyclic AMP (adenosine 3',5'-monophosphate)^{1,2}. Cyclic AMP may enhance the contraction by increasing the trans-sarcolemmal flux of Ca²⁺ during the plateau of the action potential^{3,4}, but the flux seems insufficient to activate directly the myofilaments and it is generally assumed that additional Ca²⁺ may be released from intracellular stores (ref. 5), possibly by a Ca²⁺-triggered release from the sarcoplasmic reticulum (SR)⁶⁻⁸.

Whether the intracellular level of cyclic AMP can modulate this release of Ca²⁺ is not known. Furthermore, catecholamines increase the rate of relaxation in the intact myocardial tissue⁹. How cyclic AMP could mediate this relaxing effect has not been established, although studies in isolated cardiac microsomes suggest that cyclic AMP may enhance Ca²⁺ binding by the

SR¹⁰⁻¹³ Experiments on intact cardiac tissue cannot answer these questions, as both the relaxing and inotropic effects of catecholamines and cyclic AMP might be entirely the result of the modulation of Ca^{2+} fluxes across the sarcolemma³

We removed the sarcolemma by microdissection from fragments of single cardiac cells (less than 15 μm wide and 60 μm long) obtained by the homogenisation of rat ventricular tissues⁸ (Fig 1). This allowed the cyclic AMP added to the solution to be in direct contact with the SR and the myofilaments of these 'skinned' (sarcolemma-free) cells. The resulting inotropic and relaxing effects were observed on tension recordings obtained with a highly sensitive photodiode force transducer¹⁴ (Fig 1).

The free Ca^{2+} concentration ($[\text{Ca}^{2+}]$) in the bathing solution was buffered with ethyleneglycol-bis (β aminoethyl ether) N,N'-tetraacetic acid (EGTA). The same free $[\text{Ca}^{2+}]$ was obtained with either a low or a high total [EGTA] (Fig 1).

In the presence of a slight buffering with 5×10^{-5} M total EGTA cyclic contractions were induced by the increase of free $[\text{Ca}^{2+}]$ from 2.24×10^{-8} M to 3.16×10^{-7} M (Fig 1a). The amplitude of the cyclic contractions was much smaller when the same increase of free $[\text{Ca}^{2+}]$ was associated with an increase of total [EGTA] from 5×10^{-5} M to 1.5×10^{-4} M (Fig 1b). In the presence of 2×10^{-4} M or 4×10^{-3} M total EGTA, no cyclic contractions were observed and a tonic tension was induced by the same increase of free $[\text{Ca}^{2+}]$ (Fig 1c, d and e).

This experiment suggests that the cyclic contractions were caused by cycles of release and re-sequestration of Ca^{2+} by an intracellular pool of Ca^{2+} which was in competition for Ca^{2+} with the EGTA buffer present in the solution. When the total [EGTA] is high (4×10^{-3} M) any Ca^{2+} released or sequestered by this Ca^{2+} pool would not appreciably modify the free $[\text{Ca}^{2+}]$ present in the myofilament space. Thus the observed tonic tension represented the direct effect of the free $[\text{Ca}^{2+}]$ set in the solution on the myofilaments (Fig 1d). With a total [EGTA]

just above that which inhibited the cyclic contractions (2×10^{-4} M), however, the Ca^{2+} stores were still capable of competing with the EGTA. This competition was inferred from the slower rate of tension rise and lower plateau of tension as compared with that obtained with 4×10^{-3} M total EGTA (Fig 1c, e and d).

When 10^{-6} M cyclic AMP was added to the solution with 2×10^{-4} M total EGTA, a partial relaxation of the tonic tension was obtained and was followed by the development of cyclic contractions (Fig 1f). Conversely, removal of the cyclic AMP from the solution resulted in an increase of the tonic tension and in the disappearance of the cyclic contractions (Fig 1f). Similar results were obtained in all of the 22 experiments done according to this procedure. These results indicate that cyclic AMP increased the capacity for Ca^{2+} sequestration of the internal pool of Ca^{2+} , so that it could compete with larger total [EGTA].

In the presence of a free $[\text{Ca}^{2+}]$ of 2.24×10^{-8} M with 5×10^{-5} M total EGTA, a skinned cell was quiescent (Fig 2a). A slight increase of free $[\text{Ca}^{2+}]$ to 3.98×10^{-8} M was sufficient to induce a phasic contraction. Then the total [EGTA] was increased to 4×10^{-3} M so that the direct effect on the myofilaments of the free $[\text{Ca}^{2+}]$ set in the buffer could be studied in the same cell. A free $[\text{Ca}^{2+}]$ of 3.98×10^{-8} M (the same free $[\text{Ca}^{2+}]$ as that inducing the phasic contraction with 5×10^{-5} M of total EGTA) did not evoke any tension when the total [EGTA] was 4×10^{-3} M. Thus this free $[\text{Ca}^{2+}]$ was below the threshold for direct activation of the myofilaments. A free $[\text{Ca}^{2+}]$ of 3.16×10^{-7} M with 4×10^{-3} M total EGTA induced a tonic tension smaller than the phasic contraction obtained with a free $[\text{Ca}^{2+}]$ nine times smaller. This suggests that the phasic contraction was the result of a release of Ca^{2+} from the internal stores permitting the myoplasmic free $[\text{Ca}^{2+}]$ to reach a much higher value than that set in the buffer. This release of Ca^{2+} was induced by a slight variation of free $[\text{Ca}^{2+}]$

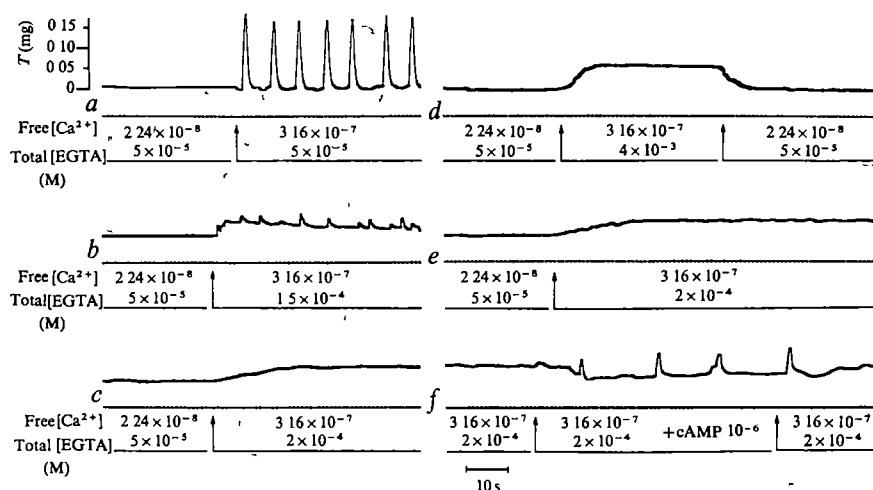


Fig 1 Tension recording in a single skinned cardiac cell of 10 μm width and 45 μm length from rat ventricle. All tracings were obtained from the same cell. Tracings d, e and f are continuous. Arrows indicate solution changes. Adult rats (200 g) were decapitated. The heart was rapidly removed and the ventricular tissue was homogenised with a Virtis blender. An aliquot of the homogenate was placed in a perfusion chamber on the temperature controlled stage of an inverted Reichert Biovert Microscope. One end of a broken cell was immobilised with a glass microelectrode, while another microelectrode was used to pull the sarcolemma and superficial myofibrils from the cell. The two ends of the skinned cell were impaled with glass microtools (heat occluded microelectrodes) in a direction perpendicular to the axis of the myofibrils. The myofibrils developed strong adherence to the glass. One of the microtools was immobilised while the other was connected to the lever of a photodiode force transducer¹⁴. Perfusion was accomplished by several inlets (one for each solution) and one suction outlet. All media contained glucose 7 mM and Tris-maleate 18 mM, pH was 7.0 and the

temperature was maintained at 22°C. The concentrations of Na_2EGTA , CaCl_2 , Na_2ATP and MgCl_2 were varied to obtain the desired free $[\text{Ca}^{2+}]$, while the free $[\text{Mg}^{2+}]$ was held constant at 3.16×10^{-4} M and $[\text{MgATP}]$ at 3.16×10^{-3} M. The ionic strength was kept constant at 0.16 M by appropriate addition of KCl. The following apparent association constants were used at pH 7.0: Ca-EGTA 4.9×10^6 M⁻¹, Mg-EGTA 40 M⁻¹, Ca-ATP 5×10^3 M⁻¹, Mg-ATP 11.4×10^3 M⁻¹. If the binding of Ca^{2+} by ATP was ignored, a simplified expression of the free $[\text{Ca}^{2+}]$ would be

$$-\log_{10} \text{free } [\text{Ca}^{2+}] \simeq 6.69 + \log_{10} \frac{\text{total } [\text{EGTA}] - \text{total } [\text{calcium}]}{\text{total } [\text{calcium}]}$$

Thus the same free $[\text{Ca}^{2+}]$ can be obtained with various levels of total [EGTA] by appropriate variations of total [Calcium]. The cyclic AMP was obtained from Sigma Chemical Co., St Louis, Missouri.

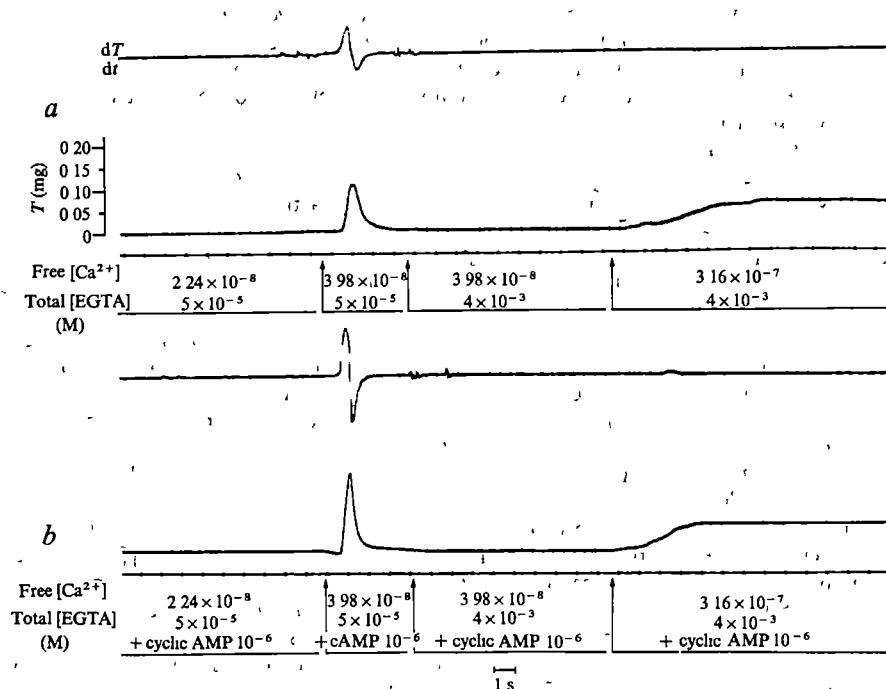


Fig. 2 Tension recording in a single skinned cell of 11 μm width and 52 μm length in the absence or in the presence of cyclic AMP. Arrows indicate perfusion changes. In addition to the experiments of the type represented in this figure, a curve of tension as a function of the free $[\text{Ca}^{2+}]$ was obtained, in the presence of 4×10^{-3} M total EGTA. The addition of 10^{-6} M of cyclic AMP did not change significantly any of the points on this curve.

in the buffer, consistent with a regenerative process⁶⁻⁸

The same series of perfusions were repeated on the same cell with the addition of 10^{-6} M cyclic AMP to the perfusion media. The resulting phasic contraction was of larger amplitude (increase of $78\% \pm 19\%$ (SD) for 18 experiments) with shorter duration (decrease of $17\% \pm 9\%$) and faster rate of tension development and relaxation than in the absence of cyclic AMP (Fig. 2b). In contrast, no modification of the tonic tension obtained in the presence of a high total [EGTA] was observed (Fig. 2b). Addition of 10^{-2} M azide (inhibitor of the respiration linked uptake of Ca^{2+} by the mitochondria) and of 10^{-6} M ruthenium red (inhibitor of the passive binding of Ca^{2+} by the mitochondria) modified neither the phasic contraction nor the effects of cyclic AMP. In contrast, the destruction of the SR by the non-ionic detergent Brij 58 (ref. 15) eliminated the phasic contraction and the effects of cyclic AMP. Therefore, the effects of cyclic AMP on the phasic contraction and relaxation of the skinned cardiac cells seem to be related to modifications in the release and binding of Ca^{2+} by the SR and not by the mitochondria.

In conclusion, cyclic AMP has been shown to modulate contraction and relaxation of single cardiac cells in the absence of the surface membrane. The relaxing effect of cyclic AMP in skinned cardiac cells is probably because of an enhancement of the capacity (Fig. 1) and the rate (Fig. 2) of Ca^{2+} binding within the SR. Additionally, the release of a larger amount of Ca^{2+} from more fully loaded Ca^{2+} stores within the SR in the presence of a higher level of cyclic AMP may explain at least in part the positive inotropic effect of catecholamines. In contrast, no direct effect of cyclic AMP on the sensitivity to Ca^{2+} of the myofilaments was shown in the present study.

A.F. is an Established Investigator of the American Heart Association.

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Electrical activity and colonial behaviour in Anthozoan hard corals

ALTHOUGH colonial behaviour in the Anthozoa¹⁻⁴ has often been described, hypotheses relating the control of colonial polyp retraction to proposed nerve net activity have been based only on behavioural observations. This preliminary report of an electrophysiological investigation considers the conduction system controlling colonial polyp retraction in the Madreporarian coral *Porites porites* var. *clavaria*.

Single electrical stimuli of short duration at threshold level did not evoke any visible withdrawal response from any polyps. Two or more such stimuli applied at a single point within a second or two, however, always caused a colonial polyp retraction (Fig. 1). The retraction response was restricted to those polyps near to the point of stimulation and reached a maximum area of spread which was not exceeded even when large numbers of stimuli were applied. A colonial polyp retraction in response to a single shock could only be evoked when stimuli of longer duration were used.

A single stimulus at threshold level evoked a single impulse which was conducted to large areas of the surrounding colony. There was no evidence for multiple firing in response to stimuli of this kind. Figure 2 shows a typical electrical response given by *P. porites*.

In the sea anemones^{5,6} and the Octocorallia⁹, available evidence suggests that the nerve net is responsible for the control of polyp retraction and that nerve/muscle potentials are the most easily recorded electrical events. This suggests that the electrical activity reported here for the colonial Hexacorallia also involves a nerve net/muscle response, although it is possible that the system may be neuroid. Electrical pulses could be stimulated by either mechanical or electrical stimuli.

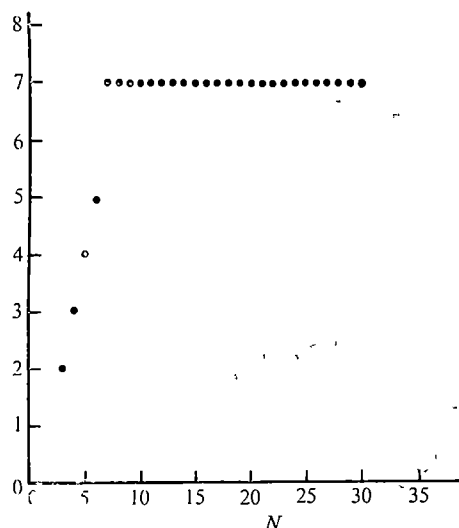


Fig. 1 Spread of colonial polyp retraction in *P. porites*, following repetitive electrical stimulation at a rate of 1 s^{-1} . r , radial distance of spread measured in polyp diameters, N , number of stimuli. Extracellular, polythene suction electrodes (see ref. 5) were used to record electrical activity. Similar electrodes were used to administer electrical stimuli. Each electrode was usually placed on the column or on the oral disk of a polyp.

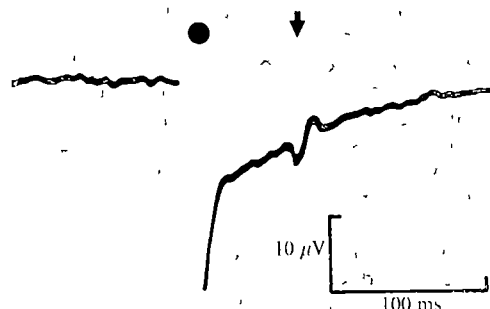
The conduction velocity in the non-fatigued conduction system was $15\text{--}20\text{ cm s}^{-1}$ at 25°C and the amplitude of the recorded pulses was small ($5\text{ }\mu\text{V}$ or less) with a threshold of $4\text{--}5\text{ V}$ for stimuli of 1 ms .

Colonial pulses are normally through-conducted, an observation at variance with predictions for many Anthozoan corals^{4,7}. These predictions were limited by the nature of the observations which could be made at that time and, based on behavioural data, assumed that nerve-net pulses did not spread significantly outside the area visibly shown to respond. The paradox that electrical activity is normally through-conducted although the spread of the behaviour is restricted, requires explanation.

Control of fast muscle contraction in the Anthozoa depends on the phenomenon of neuromuscular facilitation⁸. To bring about colonial polyp retraction, therefore, a number of pulses must be conducted across the colony. Furthermore, the frequency of the pulses will determine whether or not retraction occurs at any particular point, since this will determine the degree of facilitation of the neuromuscular junctions.

In response to a burst of stimuli at constant frequency, a corresponding burst of pulses was elicited but not at a constant frequency. It decreased in response to (1) increasing distance from the point of stimulation, (2) increasing number of pulses (Fig. 3). A number of explanations are possible for the progressively increasing conduction delays of successive pulses. If a nerve net is involved, there may have been progressively increasing synaptic delays, or the conduction path may have become longer and less direct. The reduction in frequency was most pronounced where the pulses were close together.

Fig. 2 Recording of electrical activity (arrow) in *P. porites* following a single electrical stimulus (black spot).



in time, as the frequency of pulses decreased, the rate of slowing down decreased. The changes in conduction delay could account for the restriction of the area of polyp retraction because as the frequency of pulses progressively decreases the neuromuscular facilitation resulting from the preceding pulses decays before succeeding pulses arrive.

Horridge⁴ described patterns of polyp retraction in a number of colonial Alcyonarian and Madreporarian corals. He provided behavioural evidence showing that in some species (for example, *Favia*, *Coelaria*, *Goniastrea* and *Alcyonium*) all the polyps of the colony would retract in response to stimuli applied at one point, whereas in others (for example, *Porites*), the retraction response was restricted in spread and the whole colony was never involved in a colonial retraction response stimulated from one point. From these results, he developed a theory to predict the underlying nerve net events in terms of the 'density of active units' in a large array, and the probability of crossing from one unit to another. It was suggested that the size of contraction was related to the number of 'active units' (neurones or groups of neurones) in the nerve net at that point. Thus, in a region in which no contraction would take place, there was no reason to expect any significant amount of nerve net activity.

The results reported here fail to substantiate such a model or one based on the classic ideas of interneural facilitation. The different responses may be explained, however, as a function of the amount of delay introduced between electrical events conducted across the colony. The time intervals be-

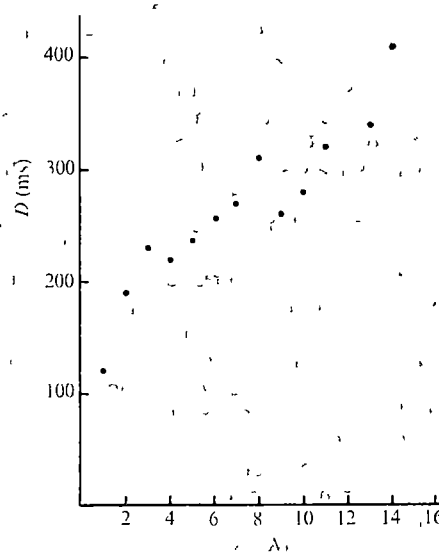


Fig. 3 Increase in conduction time with repetitive electrical stimulation at a rate of 1 s^{-1} . D , Conduction delay (ms), N , number of stimuli.

tween successive pulses determine the degree of facilitation of the neuromuscular junctions and this in turn controls the size of the muscular contraction evoked. Further data for colonial members of the sub-class Hexacorallia and for members of the sub-class Octocorallia will be reported elsewhere^{9,10}.

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Light autoradiographic localisation of cholinergic muscarinic receptors in rat brain by specific binding of a potent antagonist

THE presence of cholinergic muscarinic receptors on neurones in many regions of the mammalian brain is known from classical electrophysiological studies, which showed that neuronal firing rates are altered by the microiontophoretic application of cholinergic muscarinic agonists and antagonists¹. It is now possible to measure these receptors directly by biochemical procedures, some of which involve studying the responses of the cyclic guanosine monophosphate system in neuronal tissue to agonists and antagonists²⁻⁵. Other procedures utilised radiolabelled muscarinic agonists and antagonists that bind specifically and directly to presumed receptor sites⁶⁻¹². One of these agonists^{13,14}, 3-quinuclidinyl-benzilate (QNB) binds in a radiolabelled form to apparent muscarinic receptors *in vitro*^{6,7}. Recent experiments indicate that it is also possible to demonstrate specific ³H-QNB binding to cholinergic muscarinic receptors in rat brain *in vivo* soon after intravenous administration¹⁵. Because of this latter factor, we have been able to examine the *in vivo* localisation of ³H-QNB in regions of rat brains by light microscopic autoradiography. We have found receptor sites associated with various groups of cells, some of which are known to be cholinceptive. In general, the greatest densities of ³H-QNB sites were in telencephalic regions.

Six male Sprague-Dawley rats (180-220 g) were administered 180 μ Ci of ³H-QNB (4 Ci mmol⁻¹) by tail vein injection in 0.3 ml of saline and killed by decapitation 1 h later. Three animals were pretreated with atropine (50 mg kg⁻¹, intramuscularly), 30 min before injection of ³H-QNB. It was necessary to utilise atropine-pretreated animals since all QNB is not displaceable by muscarinic drugs *in vitro* or *in vivo*. Only the displaceable portion is pharmacologically relevant and of interest here^{6,7,15}. There was no metabolism of ³H-QNB in the brain at the time of death¹⁵. The brains were removed rapidly from the skulls and 2-5 mm slices of the appropriate regions were mounted on copper block supports and quickly frozen by partial immersion in liquid nitrogen or, in later experiments, in a propane-propylene (10/1) mixture at liquid nitrogen temperatures. Frozen coronal sections (4 μ m thick) of these tissues were thaw-mounted^{16,17} on to emulsion-coated (Kodak NTB 3) microscope slides and stored desiccated for 30 d at 2°C. After exposure, the slides were brought to room temperature, developed, fixed, immersed in Carnoy's solution (a histological fixer) and stained with pyronine Y. After drying they were mounted with Permount and observed with a Zeiss Universal microscope. Control slides prepared for positive and negative chemography showed no evidence of significant fading of latent images or spurious generation of grains after 60-d exposures. The overall autoradiographic procedure utilised in this study has been described previously¹⁶⁻¹⁸. We are also using other procedures^{17,19} which presumably would reduce further possible diffusion artefacts. Preliminary experiments, however, show results similar to those presented here. With regard to the problem of diffusion, a major factor in our favour is that the K_d for the QNB-receptor interaction is very low, 0.06 nM, and rate of dissociation of the QNB-receptor complex is correspondingly slow *in vitro*⁶ and *in vivo*¹⁵.

Of the areas examined, we found the highest densities of autoradiographic grains over the corpus striatum (nucleus caudatus-putamen), the cerebral cortex and the hippocampal

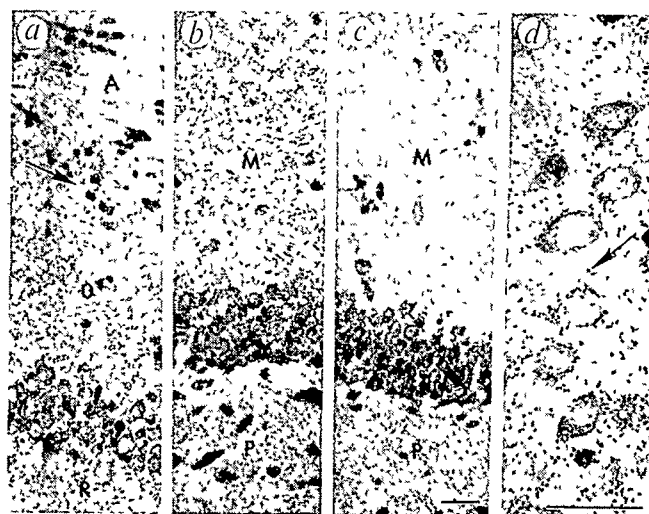


Fig 1 Light autoradiographs of regions of the brain. The grains are shown most clearly in (d) (arrow), a higher power micrograph of the striatum. Lower power micrographs are shown in (a), (b) and (c) to make the pattern of distribution more observable. Bars represent 25 μ m. a, Section of hippocampus proper showing pyramidal cells and adjacent areas in CA1. Note the high density of grains over regions on either side of the pyramidal cells, the stratum oriens (O) and the stratum radiatum (R), which contain the basal and apical dendrites of the pyramidal cells. The density decreases sharply at the boundary of (see arrow pointing along the boundary) and over the alveus (A), a fibre tract. b, Section of the dentate gyrus showing the granule cell layer and the adjacent areas, stratum moleculare (M) and stratum polymorphe (P). The stratum moleculare contains the dendritic processes of the granule cells, while the stratum polymorphe (this picture shows an area away from the pyramidal cells in CA4), is a heterogeneous zone containing the axons of the granule cells. Note the much higher density of grains in the region containing the dendritic processes. c, Same region as in (b) but from an atropine-pretreated animal. Note the great reduction in density of grains in the stratum moleculare, the dendrite-containing region while there is little or no change in the stratum polymorphe. d, Higher magnification of a section of the striatum, a region with a similar density of grains as that of the dendrite-containing regions of the hippocampus. The density was relatively uniform across the entire nucleus caudatus-putamen.

formation (Fig 1, Table 1). These results agree with our earlier *in vitro* and *in vivo* studies with rat brain^{6,15}. Apart from this more general evaluation, we were able to examine the distribution of grains in relation to specific types of cells known to have cholinergic muscarinic receptors. For example, the evidence is quite strong that acetylcholine is a neurotransmitter in septal afferents to the pyramidal and granule cells in the hippocampal formation^{20,21}. The cholinergic receptors on these cells are predominantly muscarinic, and most hippocampal cells were sensitive to acetylcholine, suggesting a widespread distribution of receptive areas²²⁻²⁴. While choline acetyltransferase and acetylcholinesterase are distributed very similarly in discrete layers in the hippocampus, acetylcholinesterase staining boutons do not seem to be layered, but are scattered diffusely throughout the tissue, suggesting that cholinergic terminals are predominantly axo-dendritic²³. Thus one would expect a widespread distribution of autoradiographic grains over regions containing dendrites of the pyramidal cells and perhaps also the granule cells. Our observations were consistent with these expectations. While these techniques do not permit visualisation of the dendritic processes, we found a high density of autoradiographic grains over the stratum oriens and stratum radiatum, regions containing the basal and apical dendrites of the pyramidal cells. The grain density seemed to be the same and relatively uniform in both of these regions, and was abruptly reduced at the boundary of and over the alveus, a fibre tract (Fig 1a). The grain density was also greatly reduced in these areas in atropine-pretreated animals, indicating that most of the ³H-QNB binding in these

areas was the specific, pharmacologically relevant binding. The stratum moleculare of the dentate gyrus, a region containing the dendritic shafts of the granule cells, also exhibited a high grain density. The density decreased abruptly at the ventral boundary of the dentate gyrus and was reduced in the stratum polymorphum, a heterogeneous zone containing the axons of the granule cells (Fig 1b). The grain density in most sections was distributed evenly across the stratum moleculare, but in more posterior sections it was much higher near the granule cell bodies and much lower in the outer regions of the stratum moleculare. The density of grains in these regions was markedly reduced in sections from rats pretreated with atropine indicating the presence of many receptors (Fig 1c). For example, when examined quantitatively, the stratum moleculare had 11.4 ± 0.65 grains per $100 \mu\text{m}^2$ in normal tissues, and 2.85 ± 0.53 grains per $100 \mu\text{m}^2$ in atropine-pretreated tissue. The background density found on the slides, off but near the tissue, was 0.33 ± 0.05 grains per $100 \mu\text{m}^2$ (data are mean \pm s.e.m. from six slides of three animals).

We also sampled several other regions of the rat brain (Table 1). In the corpus striatum, a region where most cells are sensitive to iontophoretically applied acetylcholine²⁵⁻²⁷, and where there is also a high level of muscarinic receptors^{7,12}, we observed a high density of autoradiographic grains. This density was relatively uniform and high across the entire nucleus, suggesting a lack of clustering of cholinergic cells. When examined quantitatively, the density of grains was more than four times higher between cells than over cells (12.9 grains per $100 \mu\text{m}^2$ compared with 2.96 grains per $100 \mu\text{m}^2$), again suggesting a localisation to dendritic processes (Fig 1d). The density of grains was much diminished over adjacent areas of white matter such as the corpus callosum and the bundles of internal capsule fibres. Even though the density of grains in the white matter was very low, it sometimes seemed to be reduced by atropine, in agreement with *in vitro* studies^{7,12}. It has been suggested that the binding sites in white matter areas are receptors localised on axons of cholinergic neurones¹².

In the cerebral cortex, the number of cells electrophysiologically sensitive to acetylcholine depends on the location explored^{28,29}. In this study, we observed variations in the density of grains in various regions of the cortex. There was a high density over cingulate areas and around cells in the pyriform cortex but a lower density at intermediate depths from the

surface (Table 1). In general, the density was greatly reduced in sections from atropine-pretreated animals, just as in the other regions.

The high densities observed in these regions differ from the lower density found around cells in various nuclei in the thalamus, hypothalamus, midbrain, medulla and cerebellum (Table 1). Perhaps cholinergic receptors in these regions are pharmacologically different from the QNB-type sites.

Thus it seems that one can associate these QNB binding sites with groups of cells in various brain regions. These findings would be useful for exploring the full significance and function of these presumed receptors. We are extending these studies so as to document more completely the distribution of these sites, to quantitate the distribution, and to examine the pharmacology of these presumed receptors in detail by electrophysiological methods.

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Table 1 Relative density of autoradiographic grains in representative brain regions

Region	Relative grain density
Hippocampus	
Stratum oriens	++++
Stratum radiatum	++++
Stratum moleculare	++++
Alveus	+
Striatum	++++
Cerebral cortex	
Cingulate cortex	++++
Pyriform cortex	+++
Intermediate depths of cortex	++
Thalamus	
Lateral thalamic nuclei	++
Nucleus parafascicularis	++
Medial habenular nucleus	++
Hypothalamus	
Arcuate nucleus	++
Dorsomedial nucleus pars ventralis	++
Midbrain	
Raphe nucleus	+
Interpeduncular nucleus	+
Cerebellar cortex	0 to +

Relative densities were determined by examining the brain regions after exposure for 30 d. Densities refer to specific binding as they are corrected for blank values obtained by examining similar regions from atropine-pretreated animals. Relative grain densities for cerebral cortex and striatum were measured on coronal sections of rat brain showing Brodmann's area 6 of the cerebral cortex.

Antagonism of tolazoline by histamine H₂-receptor blockers

TOLAZOLINE (2-benzyl-2-imidazoline), a peripheral vasodilator and adrenergic blocking agent used to treat peripheral vascular disease and (formerly) hypertension, has actions similar to those of histamine, including vasodilation, pressor responses in the rabbit and stimulation of intestinal smooth muscle, gastric secretion and the heart. The cardiac stimulation has usually been referred to as sympathomimetic. Tachycardia and gastrointestinal distress are prominent clinical side effects of tolazoline. It has been used in place of histamine as a diagnostic agent for gastric function^{1,2}. The effects of tolazoline on gastric acid secretion and heart rate have, however, not been explained adequately in terms of mechanism or receptor theory. We have now been able to investigate this aspect using two specific H₂-receptor antagonists³, burimamide and metiamide. Our results indicate that the actions of tolazoline on sinus rate and

gastric acid secretion can be classified as H_2 -histaminergic. Recognition that tolazoline interacts with specific histamine receptors helps to explain why the drug has so many diverse actions affecting almost every organ in the body.

To investigate the influence of H_2 -receptor antagonists on tolazoline-induced gastric acid secretion we used female beagles with chronic gastric fistulae. Dogs were fasted overnight and prepared for intravenous infusion. Tolazoline was infused throughout the experiment, and gastric juice was collected every 15 min, the volume was measured and an aliquot was titrated with base to determine acid concentration. Burimamide or metiamide was dissolved in saline containing an equivalent of HCl and administered intravenously immediately after the seventh collection period (105 min) when secretion had reached a plateau.

Metiamide and burimamide, in doses which produced no other observable effects, significantly decreased both the gastric acid concentration and the volume secreted in response to tolazoline ($20 \mu\text{mol kg}^{-1} \text{h}^{-1}$). As Fig 1 shows, metiamide ($20 \mu\text{mol kg}^{-1} \text{h}^{-1}$) promptly decreased acid secretion for about 2 h, with a peak effect of nearly complete abolition of output. Half this dose elicited a similar response lasting about 1 h with a peak of 70% reduction in acid concentration. Burimamide ($20 \mu\text{mol kg}^{-1}$) also promptly decreased secretion for about 1 h, with a peak of 80% reduction. Thus, low doses of two H_2 -receptor antagonists markedly inhibited the near-maximal effect of tolazoline on gastric acid secretion. Under similar conditions, pyrilamine, an H_1 -receptor antagonist had no

Fig 1 Effect of H_2 -receptor antagonists on gastric acid secretion induced by infusion of tolazoline in dogs. Tolazoline was infused intravenously continuously beginning at time 0 at a rate of $20 \mu\text{mol kg}^{-1} \text{h}^{-1}$ in saline (20 ml h^{-1}). Effect of metiamide, $10 \mu\text{mol kg}^{-1}$ (○) and $20 \mu\text{mol kg}^{-1}$ (●), on acid concentration (mean \pm s.e.m., $n = 4$ each dose). Effect of burimamide, $20 \mu\text{mol kg}^{-1}$, on acid concentration (mean \pm s.e.m., $n = 2$).

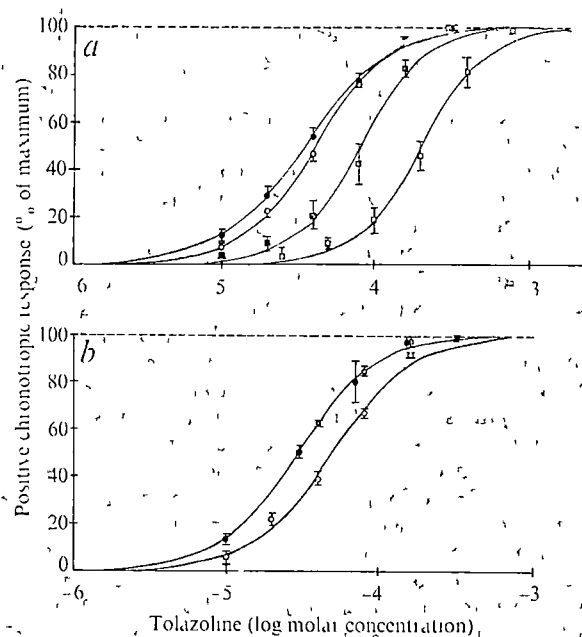
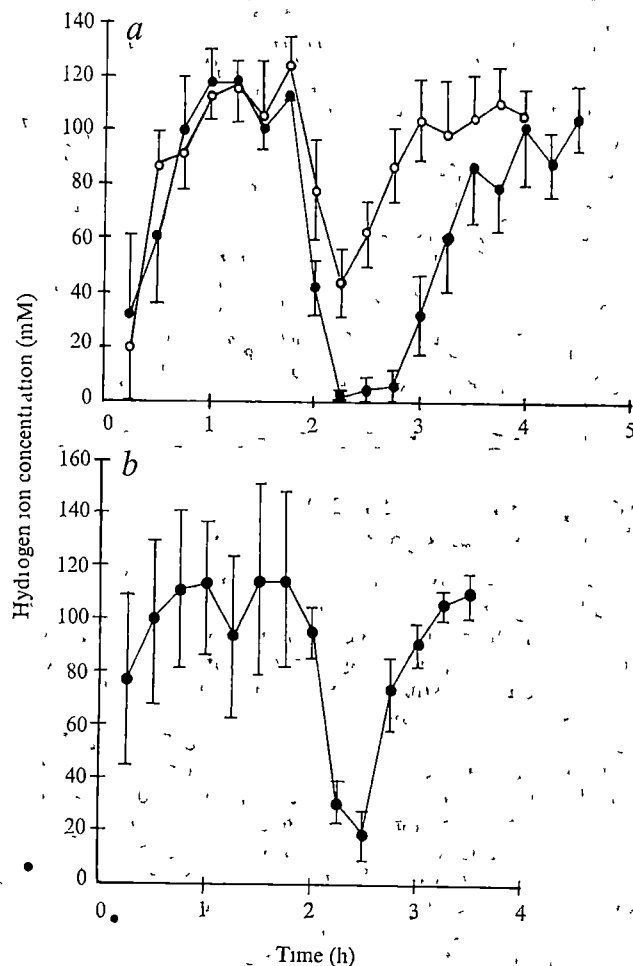


Fig 2 Effect of H_2 -receptor antagonists on the positive chronotropic response of guinea pig atria to tolazoline. The nonlinear least squares estimation program NONLIN (ref. 8) was used to fit the log dose-response curves to the logistic function⁹

$$\frac{y}{Y} = \frac{c^n}{c^n + K^n}$$

where y = response, Y = maximum response, c = drug concentration giving response y , $K = \text{ED}_{50}$, and n = numerical function of the slope of the dose-response curve at the ED_{50} level. The mean response \pm s.e.m. is plotted for each tolazoline dose. **a**, Response without antagonist (●, $n = 17$) and after equilibration with metiamide at $0.2 \times 10^{-6} \text{ M}$ (○, $n = 6$), $1.0 \times 10^{-6} \text{ M}$ (■, $n = 6$), and $4.0 \times 10^{-6} \text{ M}$ (□, $n = 6$). **b**, Response without antagonist (●, $n = 6$) and after equilibration with $2.0 \times 10^{-6} \text{ M}$ burimamide (○, $n = 6$).

effect. Our results suggest the involvement of H_2 -receptors in the secretagogue activity of tolazoline.

To investigate whether the cardiac effects of tolazoline might also be mediated by specific histamine receptors^{5,6}, and to provide more rigorous evidence on the mode of action of tolazoline, we studied the effects of tolazoline on heart rate in relation to cardiac H_2 -receptors identified by Black *et al.*³ and Parsons.⁷ We measured the activity of tolazoline on contraction frequency of spontaneously beating atrial pairs, and its antagonism by metiamide and burimamide. Male guinea pigs (500–700 g) were stunned by a blow to the head and killed by exsanguination. The heart was removed immediately and placed in oxygenated (95% O_2 , 5% CO_2) Krebs-Henseleit buffer (pH 7.4). Both atria were dissected rapidly and suspended at 1 g tension in a thermostatically controlled (30°C) tissue bath (50 ml) of the same oxygenated buffer. Tissues were allowed to stabilise, with repeated washing, for 1 h. Individual contractions were recorded with a force-displacement transducer through a strain gauge coupler, and instantaneous rates were obtained with a cardiometer. Cumulative dose-response curves were constructed after sequential additions of tolazoline as a concentrated solution in saline so that the total added volume was less than 1% of bath volume. Heart rate was allowed to stabilise before addition of each subsequent dose. After a dose-response curve in the absence of antagonist had been obtained, tolazoline was washed out until the rate returned to baseline. The procedure was then repeated after equilibration ($\geq 10 \text{ min}$) with the appropriate concentration of antagonist. The positive chronotropic response was defined as the increase over baseline rate just before addition of tolazoline. The baseline rate of 115 ± 2 beats per min ($n = 50$) was unaffected by burimamide or metiamide in the concentrations used. Maximal

rates in response to tolazoline (180 ± 3 beats min^{-1} , $n = 26$) were invariably lower than those observed with histamine under the same conditions (211 ± 3 beats min^{-1} , $n = 30$), and with tolazoline above $400 \mu\text{M}$, both rate and amplitude of contraction were depressed from their maxima. To normalise variations among tissues, responses are reported as percentage of the maximum increase, calculated for each preparation.

Metiamide, a competitive antagonist of histamine⁷, caused a parallel displacement of the tolazoline dose-response curves obtained with guinea pig atria (Fig. 2). Maximum responses were unchanged, and analysis of variance of the slope function, n , showed no significant difference in the slope of the curves. Ratios (DR) of doses needed for equal responses before and after equilibration with metiamide (Met) were calculated from the ED_{50} values and plotted according to the equation for competitive antagonism

$$\log(\text{DR}-1) = \log(\text{Met}) - \log K_B$$

where K_B = apparent dissociation constant for the antagonist-receptor complex¹⁰. The slope of the linear regression of $\log(\text{DR}-1)$ on $\log(\text{Met})$ was 1.07, not significantly different from the theoretical value of 1. The K_B for metiamide, calculated according to Waud and Parker⁹, was $8.5 \times 10^{-7} \text{M}$ ($pA_2 = 6.1$) at 30°C , in good agreement with the value obtained by Parsons⁷ ($K_B = 9.2 \times 10^{-7} \text{M}$, $pA_2 = 6.0$ at 34°C) using histamine as agonist. According to Schild's criteria for classification of receptors^{11,12}, tolazoline and histamine appear to bind to the same receptors. As a point of interest, our ED_{50} values for the positive chronotropic response to tolazoline and to histamine were $35.2 \pm 1.2 \mu\text{M}$ ($n = 17$), and $1.2 \pm 0.2 \mu\text{M}$ ($n = 24$), respectively.

Burimamide also inhibited the positive chronotropic response to tolazoline. The curves shown in Fig. 2 were constructed from data obtained in two experiments using three atrial pairs in each. Burimamide at a concentration of $2.0 \times 10^{-6} \text{M}$ caused a parallel shift to the right of the dose-response curve by a factor of 1.8 and had no effect on the maximum response. The single dose ratio calculated from these data (assuming slope = 1, $pA_2 = 5.5$ at 30°C) falls within the 95% confidence limits reported by Black *et al.*³ for burimamide using histamine as agonist.

In addition to its effect on heart rate, tolazoline increased the amplitude of contraction which was antagonised by both burimamide and metiamide. Although we did not analyse the amplitude data quantitatively, it seems likely that the positive inotropic effect of tolazoline is mediated by H_2 -receptors, as shown for histamine¹³. The actions of tolazoline on cardiac rate and contractility were unaffected by the β -adrenergic receptor blocker, propranolol (10^{-6}M), or by two H_1 -receptor blockers, diphenhydramine and pyrilamine ($5 \times 10^{-6} \text{M}$ each).

In conclusion, these studies show that in addition to its well established activity as an α -adrenergic blocking agent, tolazoline is a histaminergic agonist. The use of this classification to describe tolazoline may provide the perspective needed to understand most of its pharmacological properties including the vasodilatory effects underlying its clinical usefulness. Indeed, we have shown that its pressor activity in the anaesthetised rabbit is abolished by pyrilamine unmasking a depressor response to tolazoline which is completely blocked by metiamide¹⁴. Furthermore, in the light of our evidence, previous investigations using tolazoline may require some reinterpretation because of a failure to distinguish between the adrenergic blocking activity and the histaminergic components of its actions. This may be particularly true of experiments in which tolazoline was used as a tool to dissect the actions of other drugs or to understand the functions of the adrenergic nervous system. In such cases the results can be ambiguous or misleading since in some tissues the activation of histamine receptors produces biological effects opposite to those of adrenaline while in other instances histaminergic and adrenergic effects are similar. Finally, our findings provide the medicinal chemist with fresh

clues for the design of histamine agonists and blocking agents.

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Identification of novel high affinity opiate receptor binding in rat brain

SPECIFIC opiate receptor binding sites¹⁻³, occur in the brains of numerous vertebrates. In our initial studies⁴⁻⁶ binding seemed to involve a homogeneous population of receptors, although the biphasic degradation of receptor binding by trypsin and chymotrypsin suggested more than one site³. We have used ³H-naloxone and ³H-dihydromorphine of very high specific activity, and now report evidence for a new high affinity opiate-binding site in addition to the already reported binding site of lower affinity. The high and low affinity binding site may reflect interactions of opiates with two conformations of the opiate receptor which determine agonist and antagonist actions of the drugs.

Male Sprague-Dawley rats (ARS Sprague-Dawley, Madison Wisconsin, 180-220 g) were decapitated and their brains removed immediately. The cerebellum, which contains negligible binding¹, was excised and the remainder of the brain was placed immediately in the appropriate volume of iced standard Tris-HCl buffer (50 mM, pH 7.7 at 25°C) and homogenised with a Brinkmann Polytron for 60 s at setting 3. The homogenate was centrifuged for 20 min (49,000g, 0°C), the supernatant discarded, and pellets resuspended in the original volume of iced Tris-HCl buffer with a Brinkmann Polytron for 30 s at setting 3. Samples of 2 ml were then assayed with either ³H-naloxone (New England Nuclear Corp., 23.6 Ci mmol^{-1}) or ³H-dihydromorphine (New England Nuclear Corp., 53.6 Ci mmol^{-1}) at the stated concentrations and conditions, then filtered and counted as previously described⁵. Values are reported as stereospecific opiate binding⁵ and based on the means of triplicate determinations. All experiments were replicated at least three times. Values for the dissociation constants and number of binding sites were determined according to Klotz and Hunston⁷.

Scatchard plots of ³H-naloxone binding both in the presence and absence of sodium describe curves which can be resolved into two linear components with K_D values of 0.4 nM and 30 nM for high and low affinity binding respectively (Fig. 1a). Previously we reported that sodium increases the binding of ³H-naloxone and decreases the binding of ³H-dihydromorphine^{6,8}. When 100 mM sodium was included in the incubation medium, the number of apparent high affinity binding sites almost doubled, but the

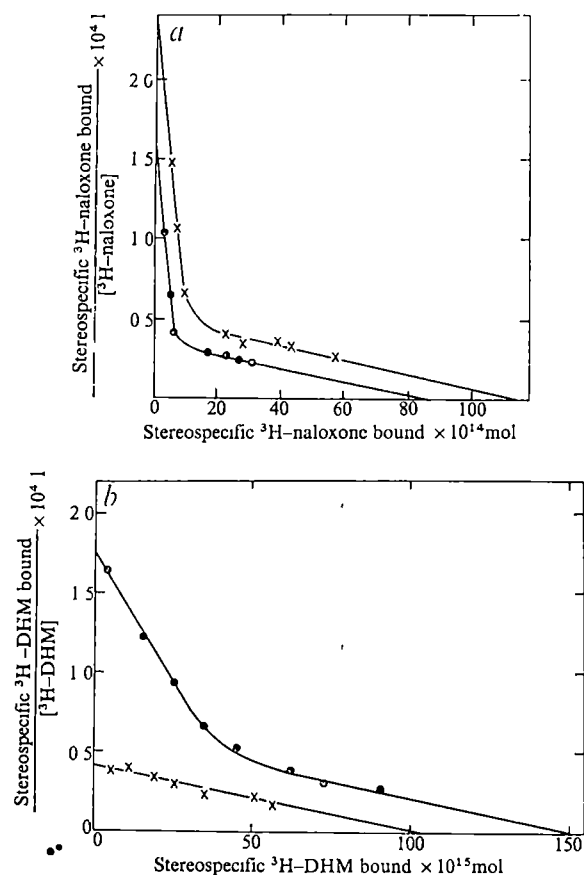


Fig 1 Scatchard plots of ^3H -naloxone and ^3H -dihydromorphine binding in the presence and absence of NaCl. *a*, ^3H -naloxone Scatchard plot. 2-ml samples of brain homogenate (100 vol) were assayed in triplicate as described in the text, with various concentrations of ^3H -naloxone in the presence (\times) or absence (\bullet) of 100 mM NaCl and either (—) or (+) 3-hydroxy-N-allyl-morphinan at $0.1 \mu\text{M}$. *b*, ^3H -dihydromorphine Scatchard plot. 2-ml samples of brain homogenate (100 vol) were assayed in triplicate, as described in the text, with various concentrations of ^3H -dihydromorphine in the presence (\times) or absence (\bullet) of 100 mM NaCl and either (—) or (+) 3-hydroxy-N-allyl-morphinan at $0.1 \mu\text{M}$. All values for binding are stereospecific.

number of low affinity sites was unaffected and there was no significant effect on the dissociation constants. The increase in ^3H -naloxone binding elicited by sodium resembled our previous observations^{8,9}.

Biphasic Scatchard plots were also obtained for ^3H -dihydromorphine binding (Fig 1 *b*). In the absence of sodium, high affinity binding for ^3H -dihydromorphine had a dissociation constant of about 0.3 nM, and low affinity binding exhibited a dissociation constant of about 3 nM. Sodium (100 nM) virtually abolished the high affinity binding of ^3H -dihydromorphine but had no apparent effect on the low affinity binding sites. Thus the effects of sodium on the binding of opiate agonists and antagonists are exerted predominantly on high affinity binding components.

The affinity of a ligand for its receptor is determined in part by its rate of dissociation from the receptor so that one would predict a slower dissociation rate for the novel high affinity binding site described here than was obtained for the lower affinity binding site reported previously⁴. Accordingly, after labelling the receptor with low concentrations (1 nM) of ^3H -naloxone we measured its dissociation from the receptor. Following the standard incubation the homogenate was centrifuged (49,000g for 20 min) and the pellet suspended in 10 times the original volume with $1 \mu\text{M}$ unlabelled naloxone incubated for various intervals at 0°C , filtered and counted. The half-life for dissociation of

naloxone was 30 min in contrast to a 5-min half-life reported at this temperature with higher concentrations of ^3H -naloxone labelling the 'low' affinity binding site⁴. The slower dissociation rate of lower concentrations of ^3H -naloxone is consistent with labelling of a novel binding site with high affinity.

In this study, using ^3H -naloxone and ^3H -dihydromorphine of high specific activities, we have detected a new opiate binding component with 100 times the affinity of ^3H -naloxone-binding sites described earlier^{4,5}. This high affinity binding, detected by using low concentrations of ^3H -opiates and ^3H -opiate antagonists, displays the same pharmacological specificity as binding using higher ^3H -drug concentrations^{1,4}.

What might be the functional significance of the high and low affinity opiate binding sites? We propose that they represent binding of opiates to two distinct conformations of the opiate receptor described recently⁸⁻¹⁰. The ability of sodium to increase binding of opiate antagonists and to decrease the binding of opiate agonists to the same extent implies that the opiate receptor exists in two forms. Antagonists have greater affinity for the antagonist-sodium form of the receptor while agonists bind preferentially to the agonist-no sodium receptor state. Pharmacological actions such as analgesia occur only when a drug binds to the agonist state of the receptor so that antagonists block the effects of opiate analgesics by binding to the antagonist conformation of the receptor, reducing the number of agonist forms of the receptor. The data presented here suggest that the 'high affinity binding' of ^3H -naloxone involves the antagonist conformation of the receptor while low affinity ^3H -naloxone binding takes place to the agonist state of the receptor. Conversely, high affinity and low affinity ^3H -dihydromorphine binding respectively involve the agonist and antagonist states of the opiate receptor.

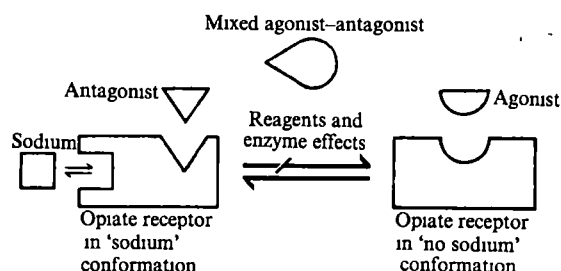


Fig 2 A model of opiate receptor function.

While binding to the agonist form of the receptor is impaired by sodium, binding to the antagonist form should be unimpaired or increased. ^3H -dihydromorphine binding to high affinity sites is greatly reduced by sodium while its binding to low affinity sites is unaffected, which confirms our suggestion that low affinity binding of ^3H -dihydromorphine involves the antagonist state of the receptor. Our inability to demonstrate a marked sodium-induced enhancement in low affinity ^3H -dihydromorphine binding and a reduction in low affinity ^3H -naloxone binding may be the result of difficulty in measuring binding at these high ligand concentrations because of the high nonspecific binding and the difficulty in separating the high and low affinity binding sites by Scatchard analysis⁷.

We reported that several protein modifying reagents which attack sulphhydryl, tryptophan and other residues selectively decrease receptor binding of opiate agonists at low concentrations which do not affect antagonist binding^{9,10}. These reagents seem to interfere with the interconversion of agonist and antagonist conformation of the opiate receptor so that it freezes in the antagonist con-

formation. If, as postulated here, high and low affinity binding of ^3H -dihydromorphine respectively involve agonist and antagonist states of the receptor, protein modifying reagents should selectively impair high affinity binding of ^3H -dihydromorphine with no effect on low affinity binding. To test this idea, we examined the influence of iodoacetamide on ^3H -dihydromorphine binding at concentrations that reduce agonist but not antagonist receptor binding. In confirmation of this prediction, iodoacetamide markedly lowered high affinity binding of ^3H -dihydromorphine with no effect on its low affinity binding.

In summary, high and low affinity binding to the opiate receptor may reflect discrete interactions with the two conformational states of the receptor that account for the pharmacological properties of agonists and antagonists. The two binding sites may provide a valuable tool to investigate opiate receptor conformations.

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Prevention of the effects of fentanyl by immunological means

INVESTIGATIONS have demonstrated that the pharmacological effects of drugs such as digitalis¹ and paraoxon² can be altered by immunological means. To date, however, all reported attempts to antagonise immunologically the *in vivo* pharmacological effects of narcotic analgesics (such as morphine) have resulted only in mild attenuation or a partial delay in the onset of action³⁻⁵. To prevent immunologically the effect of a pharmacologically active molecule, sufficient amounts of specific antibodies must be present so that less than a threshold dose of the free drug remains after combination with the antibodies. Such a condition would be maximised with a drug which can be rendered highly immunogenic and which can exert its pharmacological effect at very low concentrations. We report that it is feasible to prevent the pharmacological effects of the potent narcotic analgesic fentanyl in experimental animals by both passive and active immunisation.

We have previously reported^{6,7} that high titres of antibodies of high specificity and affinity could be induced against the very potent synthetic narcotic analgesic, fentanyl. Fentanyl [N-(1-(2-phenethyl-4-piperidinyl) propionanilide)] has a spectrum of pharmacological effects similar to morphine, all of which can be antagonised by naloxone, however, fentanyl produces pronounced analgesia at 1/200 the dose of morphine.

Fentanyl, therefore, seemed to be a good candidate for investigating the neutralisation of the effects of an opiate-like drug by antibodies.

Antifentanyl antibodies were produced by immunisation of two rabbits with carboxyfentanyl [N-phenyl, N-4(1(β -phenethyl) piperidine succinamic acid)] conjugated to the protein carrier bovine gamma globulin (BGG). Conjugation was performed using 1-ethyl-3-(3-dimethylamino-propyl)-carbodiimide as previously described⁸, the conjugate containing 12 mol fentanyl per mol BGG. Immunisation was achieved by three bi-weekly subcutaneous injections of 2 mg conjugate in saline emulsified with an equal volume of Freund's complete adjuvant (FCA). The antigen binding capacity of the antisera produced was such that 0.25 μl serum could bind 0.005 μg and 0.0025 μg ^3H -fentanyl, respectively.

Table 1 Prevention of fentanyl analgesic effect by neutralisation of the drug *in vitro* and by passive and by active immunisation

Treatment	No. of mice	Hot plate end points (s \pm s.e.m.)	
		Before treatment	90 s after treatment
Neutralisation*			
Fentanyl+saline	5	9.8 \pm 0.99	> 60
Fentanyl+control serum	5	8.6 \pm 0.99	> 60
Fentanyl+immune serum	5	6.8 \pm 0.62	7.7 \pm 1.62
Passive immunisation†			
Control serum	5	7.0 \pm 0.57	> 60
Immune serum followed by fentanyl	5	7.4 \pm 0.75	7.8 \pm 1.12
Active immunisation‡			
Immunisation with fentanyl-BGG conjugate in FCA	15	8.0 \pm 0.49	18.8 \pm 5.6
Immunisation with FCA only	10	6.8 \pm 0.54	> 60

*Fentanyl was administered at a dose of 100 μg kg^{-1} in a total volume of 0.2 ml per 30 g mouse. Fentanyl was incubated with saline, control serum or immune serum for 10 min at room temperature before intravenous administration. Saline, control serum and immune serum, when injected alone to control mice, did not alter the end point.

†Mice were given 0.5 ml control serum or immune serum intravenously and challenged with 100 μg kg^{-1} fentanyl (0.2 ml per 30 g mouse) 30 min later. Control serum and immune serum, when injected alone to control mice, did not alter the end point.

‡Specific immunisation was performed with fentanyl-BGG conjugate in Freund's complete adjuvant (FCA), controls were immunised with saline in FCA. Injections were given intraperitoneally, twice at 2 weeks interval. Challenge with 100 μg kg^{-1} fentanyl in a total volume of 0.2 ml per 30 g mouse was performed 14 d after the last injection.

Assessment of the ability of rabbit antifentanyl antibodies to bind *in vitro* with the drug and to neutralise its analgesic effect was carried out as follows. An amount of fentanyl citrate equal to three times the ED_{50} dose ($3 \times 33 \mu\text{g}$ kg^{-1}) was preincubated for 10 min with 0.3 ml specific rabbit antisera, 0.3 ml of rabbit antiserum to tobacco mosaic protein, serving as control serum, or with 0.3 ml saline and then injected intravenously to NAMRU mice⁹. The mice were subsequently tested for analgesia by the hot plate technique of Eddy and Leimbach⁹ using their criteria for end point determinations. Each mouse was tested on the hot plate before, and 90 s after intravenous injection. The results of these experiments show that intravenous administration of 3ED_{50} of fentanyl, either in saline or preincubated with control serum, produced analgesia in 100% of the tested mice. In contrast, when 3ED_{50} of fentanyl were preincubated with specific rabbit antifentanyl serum the analgesic effect of fentanyl was dramatically reduced (Table 1). We then ascertained whether prevention of drug effects could be accomplished by passive immunisation *in vivo*. Two groups of mice were given intravenous injections of either 0.5 ml of the rabbit antifentanyl serum or 0.5 ml control serum, 30 min

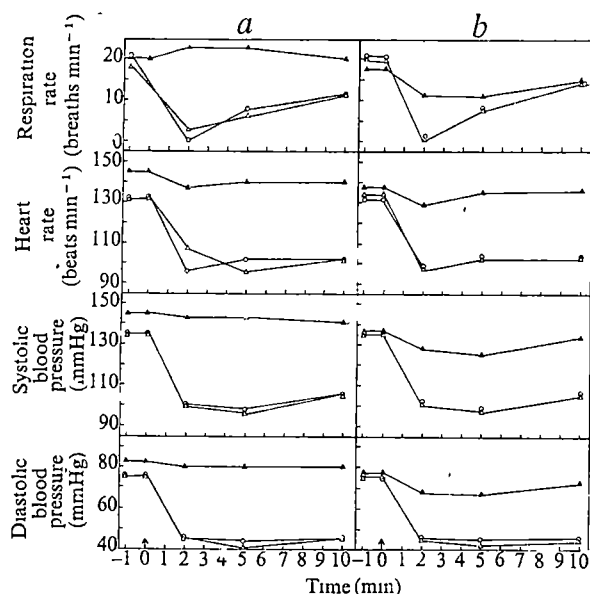


Fig 1 Prevention of the effect of fentanyl in dogs *a*, *In vitro* neutralisation $5 \mu\text{g kg}^{-1}$ fentanyl were preincubated with 2 ml saline (\circ), 2 ml control globulins (Δ) or 2 ml antifentanyl globulins (\blacktriangle) for 10 min at 37°C . The mixtures were given intravenously to two dogs at zero time (\longrightarrow) *b*, Passive immunisation two dogs were injected intravenously with 2 ml saline (\circ), 2 ml control globulins (Δ) or 2 ml immune globulins (\blacktriangle) 30 min before challenge with $5 \mu\text{g kg}^{-1}$ fentanyl at zero time (\longrightarrow)

later each mouse was challenged intravenously with 3ED_{50} of fentanyl. Table 1 shows that the analgesic effect of fentanyl was prevented only in mice receiving specific rabbit antifentanyl serum, the remaining animals exhibited analgesia. To determine whether active immunisation of mice would alter the analgesic effect of fentanyl, we immunised two groups of mice, one group receiving two intraperitoneal injections, 14 d apart, of $250 \mu\text{g}$ fentanyl-BGG conjugate in 0.1 ml saline emulsified with equal volume of FCA, the second receiving two identical injections of saline emulsified with FCA. Ten days after the second injection all the animals were bled from the tail vein, sera were collected and titres of antifentanyl antibodies determined by the Farr assay¹⁰ as follows: various dilutions of pooled sera were incubated for 60 min at room temperature with $0.01 \mu\text{g}$ of internally labelled ^3H -fentanyl. The complex was precipitated at 50% saturation of ammonium sulphate, washed three times with 50% saturated ammonium sulphate, the precipitate taken up in 10 ml scintillation fluid and the radioactivity determined on the Nuclear Chicago Mark I scintillation counter. The fentanyl-BGG-immune sera had titres to the extent that 1 μl could bind $0.0075 \mu\text{g}$ ^3H -fentanyl. Four days after serum collection the mice were challenged intravenously with 3ED_{50} of fentanyl. Table 1 shows that active immunisation prevented the analgesic response in mice challenged with 3ED_{50} of fentanyl.

To determine whether drug effects other than analgesia could also be prevented by antifentanyl antibodies, we monitored systolic blood pressure, diastolic blood pressure, respiration rate and heart rate in dogs. Preliminary experiments with two dogs weighing approximately 10 kg each showed that the intravenous administration of $2.5 \mu\text{g kg}^{-1}$ fentanyl produced marked effects on the selected parameters. Two other dogs were anaesthetised with halothane, and maintained at a minimal stable level of anaesthesia with 1.5% halothane. Neutralisation of the effects of fentanyl was attempted by preincubation of $5 \mu\text{g kg}^{-1}$ fentanyl with 2 ml control globulins, antifentanyl globulins or saline, followed by intravenous administration of the various mixtures into each of the dogs. Figure 1*a* shows that all the effects of the drug were prevented by antifentanyl antibodies, whereas the effects of the drug were fully expressed

(even to the extent that breathing had to be assisted) in the controls.

Passive immunisation was then attempted by administering 2 ml of antifentanyl globulins to two mongrel dogs (10 kg body weight) and then challenging 30 min later with $5 \mu\text{g kg}^{-1}$ of fentanyl. Figure 1*b* shows that only the administration of antifentanyl globulins had the capacity to prevent the effects of the challenging dose of fentanyl. Previous administration of either saline or control globulins had no effect on subsequent fentanyl administration.

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Liver tryptophan pyrrolase activity and metabolism of brain 5-HT in rat

TRYPTOPHAN is the amino acid precursor of 5-hydroxytryptamine (5-HT) both peripherally and in the central nervous system, but the metabolism of tryptophan by way of the 5-HT pathway accounts for only 1% of urinary tryptophan metabolites¹. The major route of tryptophan metabolism starts with its conversion to formylkynurenine by tryptophan pyrrolase², and it has been suggested^{3,4} that depressive illness may be caused by the induction of liver tryptophan pyrrolase by plasma corticosteroids⁵, leading to decreased synthesis and turnover of 5-HT in the brain. We confirm the increase in activity of liver tryptophan pyrrolase produced by hydrocortisone and the decrease in activity by allopurinol reported by Curzon and Green². Hydrocortisone also increased the concentration of 'free' tryptophan in the serum. Although the concentration of 5-HT in the brain was unchanged, tryptophan and 5-hydroxyindole acetic acid (5-HIAA) concentrations in the brain were also increased. 'Free' and total tryptophan concentrations in the serum were reduced by allopurinol injection, but concentrations of tryptophan in the brain were unchanged. These results are not compatible with the theory that the induction of liver pyrrolase by hydrocortisone induces the symptoms of depression.

Male Sprague-Dawley rats (250–300 g) were housed at constant temperature under a 12 h light/12 h dark cycle. The effects of the injection of hydrocortisone (15 mg kg^{-1}) and allopurinol (20 mg kg^{-1}) on liver tryptophan pyrrolase activity were measured and related to changes in 'free' and total tryptophan concentrations in the serum, brain tryptophan, 5-HT and 5-HIAA concentrations (Table 1). Induction of the enzyme by hydrocortisone was maximal 3 h after injection¹. Total tryptophan concentrations in the serum remained

Table 1 Effect of hydrocortisone and allopurinol on pyrrolase activity and tryptophan metabolism

	Pyrrolase activity (μmol kynurenine $\text{h}^{-1} \text{g}^{-1}$)	Serum total tryptophan (μg ml^{-1})	Serum free tryptophan (μg ml^{-1})	Brain tryptophan (μg g^{-1})	Brain 5-HT (ng g^{-1})	Brain 5-HIAA (ng g^{-1})
Control	5 84 \pm 0 38 (6)	12 61 \pm 1 06 (6)	2 05 \pm 0 18 (6)	1 42 \pm 0 06 (6)	444 \pm 8 (8)	267 \pm 9 (5)
Hydrocortisone (15 mg kg^{-1} intraperitoneally)	16 44 \pm 0 55 (6) [†]	11 82 \pm 0 54 (6)	4 81 \pm 0 97 (6)*	1 64 \pm 0 06 (6)*	407 \pm 16 (10)	321 \pm 12 (16)*
Control	6 73 \pm 0 45 (4)	11 02 \pm 0 5 (6)	3 69 \pm 0 03 (6)	1 61 \pm 0 07 (6)		
Allopurinol (20 mg kg^{-1})	2 67 \pm 0 43 (4)*	6 33 \pm 0 3 (6) [†]	2 01 \pm 0 11 (6) [†]	1 43 \pm 0 04 (6)*		

All animals were killed at approximately the same time of day to avoid the influence of the circadian variation of tryptophan, 5-HT and 5-HIAA in rat brain and serum. Total tryptophan in serum and brain was assayed by the method of Denckla and Dewey⁶, unbound tryptophan was assayed by the same method following equilibrium dialysis against Krebs-Ringer solution (pH 7.4). 5-HT was assayed by the method of Snyder *et al.*⁷, and 5-HIAA by the method of Giacalone and Valzelli⁸. Liver tryptophan pyrrolase activity was estimated by the method of Knox and Auerbach⁹.

* Significance at $P < 0.01$ level, [†] significance at $P < 0.001$ level.

Means \pm s.e. are shown. Numbers of animals per group in parentheses.

unchanged but concentrations of 'free' tryptophan in the serum and brain tryptophan were significantly increased ($P < 0.001$). Brain 5-HT concentrations were unchanged but brain 5-HIAA concentrations were significantly increased ($P < 0.01$).

Liver tryptophan pyrrolase activity was significantly decreased ($P < 0.001$) by the injection of allopurinol (20 mg kg^{-1}) intraperitoneally. Concentrations of total tryptophan in the serum were simultaneously reduced by 60% and of 'free' tryptophan by 53%. The concentrations of tryptophan in the brain were also lowered.

When allopurinol and hydrocortisone were administered together the increased pyrrolase activity induced by hydrocortisone was reversed. No significant change in concentrations of tryptophan in the brain was measured but total and 'free' tryptophan in the serum were reduced by 60% and 30% respectively.

Corticosteroids have been shown to elevate free amino acids in the plasma as a result of protein breakdown in tissues other than the liver⁵, and to enhance synthesis of liver protein including the synthesis of liver tryptophan pyrrolase⁹. Concentrations of total and 'free' tryptophan in the plasma may therefore be expected to depend on the balance of these systems. Knott and Curzon⁹ have shown that the concentrations of tryptophan in the brain are correlated with concentrations of 'free' tryptophan in the plasma. Our results agree with this latter correlation since the concentrations both of 'free' tryptophan in the serum and of tryptophan in the brain were increased after hydrocortisone injection but total tryptophan concentrations remained unchanged. Since the concentrations of tryptophan in the brain are below the K_m for tryptophan hydroxylase¹⁰ it is not surprising that the increased concentrations of brain tryptophan led to an increased turnover of 5-HT in the brain.

The hypothesis developed by Curzon³ maintained that low concentrations of 5-HT in the brain were related to increased pyrrolase activity which in turn was caused by high corticosteroid concentrations. This hypothesis depends on the assumption that the induction of liver pyrrolase by corticosteroids results in a decreased availability of tryptophan for conversion to 5-HT in the brain. The effects of hydrocortisone demonstrated in these experiments make this theory untenable, although the possibility that corticosteroids may participate in altering the metabolism of 5-HT in the brain cannot be discounted, particularly in light of recent evidence which suggests the presence of a pyrrolase enzyme in the brain¹¹.

It is also possible that, as suggested by Curzon, long term activation of pyrrolase could cause a functional deficiency of pyridoxal in the brain, or that elevated levels of tryptophan

metabolites formed on the pyrrolase pathway could have secondary effects on 5-HT levels¹².

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Increased UDP kinase activity in rat and human hepatomas

PREVIOUS work¹⁻⁴, (carried out in this laboratory with the molecular correlation concept as a conceptual and experimental approach resulted in an insight into the biochemical strategy of the cancer cell) much of it conducted with a model system of hepatomas of very different growth rates⁵, has revealed a gradually emerging metabolic imbalance that is linked with the increase in tumour growth rate^{1,4,6-12}. Such biochemical alterations were manifested in progressive changes in the activities of opposing and competing key enzymes and opposing and competing metabolic pathways that correlated closely with tumour growth rate. These alterations in gene expression that seem to be linked with the increase in the expression of malignancy were manifested in increases in the activities of key glycolytic, pyrimidine and DNA synthesising enzymes^{4,7-12}. Concurrently, there were progressive decreases in the activities of the key enzymes of gluconeogenesis, pyrimidine catabolism and the urea cycle^{4,7,8,10,12}. In addition to this 'malignancy-linked' (growth-rate-linked) metabolic imbalance, we now show that the reprogramming of gene expression in cancer cells entails 'transformation-linked' alterations that are present in all hepatomas irrespective of growth rate or differentiation.

Our investigations on the UTP-synthesising enzyme, UDP kinase (nucleosidediphosphate kinase, ATP UDP phosphotransferase, EC 2 7 4 6) were undertaken because in the hepatomas of different growth rates the capacity of certain key enzymes involved in the biosynthesis of UDP increased in parallel with cell proliferation rate¹³⁻¹⁵. Concurrently, utilisation of UDP was potentiated by an enhanced activity of ribonucleoside-diphosphate reductase (EC 1 17 4 1)⁹. Quantitatively smaller increases were also observed in differentiating and regenerating liver^{11,13} (for reviews see refs 11 and 14-16). Since the reductase and the UDP kinase compete for the UDP pool, their strategic metabolic positioning suggests that a critical imbalance in the reprogramming of gene expression may be present in the spectrum of hepatomas of different growth rates (Fig 1). Our studies with various hepatomas of vastly different growth rates and differentiation showed that specific activities of UDP kinase were markedly increased in all neoplasms irrespective of tumour proliferation rate or degree of differentiation. This increased potential for UTP biosynthesis should provide a mechanism by which the cancer cell can adjust the utilisation of UDP for RNA and deoxyribonucleotide biosynthesis for subsequent formation of DNA.

Male Buffalo and ACI/N rats were maintained as described previously^{4,7}. Preparation of regenerating liver, studies on developing animals, killing of rats and excision of livers and tumours were as reported elsewhere^{4,7,12}. The growth rates of the various hepatoma lines ranged from 2 weeks to 11.5 months, measured by the time required for the tumours to reach a diameter of 1.5 cm.

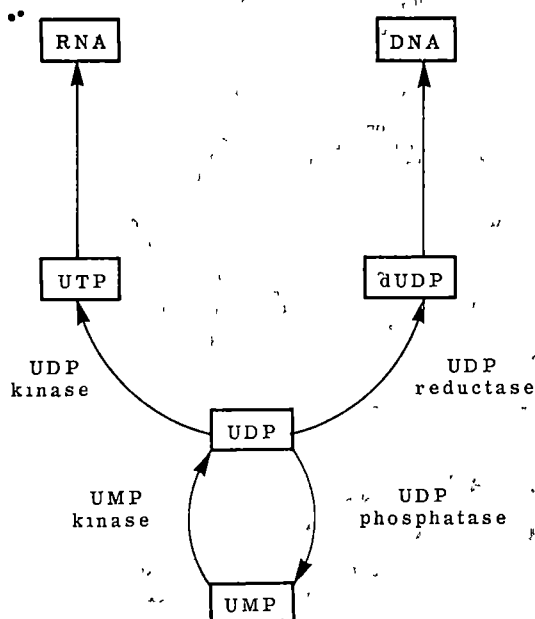


Fig 1 UDP metabolism. This simplified schematic diagram indicates the strategic position of UDP in pyrimidine, RNA and DNA metabolism. The role of the four enzymes involved in UDP production and utilisation is noteworthy. For further details on the behaviour and interrelationships of the key enzymes and metabolic pathways in liver neoplasia, see review by Weber⁴.

Kinetic studies were carried out to ensure proportionality of UDP kinase activity with amount of enzyme added. A standard enzyme assay system was developed that was adapted to the kinetic conditions of the rat liver and hepatoma systems. UDP kinase specific activity was expressed as μmol of substrate metabolised $\text{h}^{-1} \text{mg}^{-1}$ protein. The details of the assay and a comparison of the specific activity and protein content are given in Fig 2.

The kinetic characteristics of UDP kinase activity from crude enzyme preparations of rat liver and the rapidly growing hepatoma 3924A were similar. The pH optima were broad,

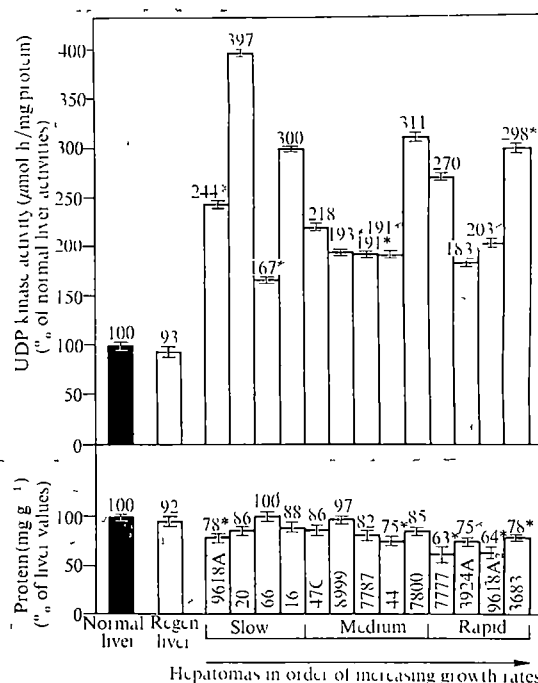


Fig 2 Increased uridine-5'-diphosphate kinase (UDP kinase) activity in rat hepatomas of different growth rates. One per cent homogenates (w/v) were prepared at 4°C from liver and tumour tissues in 0.15 M KCl, pH 7.4, which were made 5 mM dithioerythritol (DTE) after protein determination. Supernatant fluids (100,000g) were used for the determination of UDP kinase activity as measured by the conversion of ^3H -UDP to ^3H -UTP. The incubation mixture contained in a total volume of 0.2 ml, 50 μmol of glycyl-glycine, pH 7.5, 7.5 μmol of MgCl_2 , 1.5 μmol of β -mercaptoethanol, 7.5 μmol of ATP (diNa), pH 7.4, 2.5 μmol of UDP, 1.5 μCi of uridine-5- ^3H -5'-diphosphate ammonium salt (19.6 Ci mmol^{-1}), and 2 μl of a 1:4 dilution of 1% supernatant fluid. Enzymatic activity was determined by withdrawing three 30- μl samples at 2 min intervals and spotting directly on PEI-cellulose plates (Brinkmann), activated with 10% NaCl, at 65°C (hot air blower). The PEI-cellulose plates were washed for 10 min in 500 ml of anhydrous methanol and air dried. The product of the reaction (UTP) was separated from the substrate (UDP) chromatographically with 4.0 M HCOONa , pH 3.4, in glass rectangular tanks. No nucleoside-diphosphate (EC 3.6.1.6) activity was detected under these conditions. In this system the UTP spot was located (at the origin) with ultraviolet light, marked with a soft lead pencil, cut out with scissors and placed in counting vials with 10 ml of scintillation fluid (0.03% *p*-bis (2,5-phenyloxazolyl) benzene and 5% 2,5-diphenyloxazole in toluene from New England Nuclear Corp). ^3H -UTP was determined in a Packard Tri-carb (Model 3390) liquid scintillation spectrometer. Protein concentration was assayed by the Folin method of Lowry *et al*¹³, using recrystallised bovine serum albumin as a standard. One unit of UDP kinase activity will produce 1 μmol of UTP in 1 h at 37°C and pH 7.4. Specific activity was expressed as units per mg protein. UDP kinase activities of the hepatomas are compared with corresponding control liver values. The UDP kinase activity of the normal liver was 444 ± 9 μmol per h per mg protein. The data are means \pm s.e. of four or more animals in each group and the results are plotted as percentages of corresponding control liver values. The protein content was calculated as mg protein per gramme wet weight of tissue and it is also given in percentages. The measurement of growth rates of the individual hepatoma lines was discussed elsewhere⁴.

*Values statistically significantly different from the respective controls ($P < 0.05$).

ranging between 7.0 and 9.0. The apparent K_m values for UDP, ATP and MgCl_2 at pH 7.4 and 37°C were 0.5, 3.0 and 3.0 mM, respectively.

UDP kinase specific activities were markedly increased in all neoplasms irrespective of tumour growth rate or degree of differentiation. The specific activities in the slowly growing hepatomas 9618A, 20, 66 and 16 were 2.4, 3.9, 1.7 and 3.0 times greater, respectively, than those in the corresponding normal rat livers. In hepatomas of medium growth rates 47C, 8999, 7787, 44 and 7800 the activities were 2.2, 1.9, 1.9, 1.9 and

3.1 times higher than those in normal liver. In the rapidly growing neoplasms 7777, 3924A, 9618A2 and 3683, activities were 2.7, 1.8, 2.0 and 2.9 times greater, respectively, than those of normal liver.

In the 24-h regenerating liver, UDP kinase activity was similar to that in liver of sham-operated control rats. Enzyme activity in the average cell of 6-d-old rat liver was in the same range as that of the normal rat (data not shown). Since increased UDP kinase activity was present in the hepatomas, but not in rapidly growing differentiating or regenerating liver, the higher activity of this UTP-synthesising enzyme seems to be specific to neoplastic transformation. This tentative conclusion was supported by studies on two primary human hepatomas. Using the histologically normal samples from the human host liver as controls, we observed that UDP kinase specific activity in the two human hepatomas was increased 2.3 and 4.9 times.

Our studies demonstrate a marked imbalance in hepatoma UDP utilisation. Whereas UDP kinase activity was increased in all hepatomas irrespective of the rate of cell proliferation (Fig. 2), the ribonucleoside-diphosphate reductase activity from the very low levels of the liver (less than 2 pmol per h per mg protein) increased markedly in parallel with tumour growth rate⁹. Thus, the regulatory mechanisms for gene expression are reprogrammed differently for these two UDP-utilising enzymes. The reductase activity, as it is increased in parallel with the growth rate, is linked with the different degrees in the expression of malignant properties. In contrast, the increased UDP kinase activity, as it is present in all hepatomas, even in the most slowly growing well differentiated neoplasms, seems to be linked with the neoplastic transformation *per se*.

Our demonstration of high UDP kinase activity in hepatomas, irrespective of the degree of malignancy, differentiation or growth rate, suggests that the reprogramming of gene expression in malignant transformation is linked with an increase in the expression of this UTP-synthesising enzyme activity. In this laboratory recently there were discovered four such transformation-linked increases in enzyme activities, and they all relate to an increased potential in the routing of precursors to strategic biosynthetic processes. Thus, the increase in the activities of glucose-6-phosphate dehydrogenase (EC 1.1.1.49) and transaldolase (EC 2.2.1.2) provides an increased potential for channelling glycolytic intermediates into pentose phosphate biosynthesis¹⁷. The third alteration observed in all hepatomas was an increase in amidophosphoribosyltransferase (EC 2.4.2.14) activity which should also result in an increased potential for purine and nucleic acid biosynthesis¹⁸. In turn, the greater UDP kinase activity reported here should provide an increased potential for RNA and DNA biosynthesis.

These increases in the activities of key enzymes of ribose-5-phosphate biosynthesis, purine production and the increase in UDP kinase activity indicate a reprogramming of gene expression that should confer selective biological advantages to the neoplastic cells.

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Role of precursor 16S RNA in assembly of *E. coli* 30S ribosomes

RECONSTITUTION of bacterial ribosomes occurs *in vitro* in a system containing ribosomal components only¹, which suggests that ribosomes also form by self-assembly *in vivo*. This inference is reinforced by the fact that intermediary particles, containing a similar fraction of ribosomal proteins, are observed both *in vitro* and *in vivo*¹⁻³. The *in vitro* reconstitution process, however, requires a very high energy of activation, presumably to force the intermediates into a rare conformational state^{1,4}. This requirement is too high to be compatible with the rate of ribosome formation *in vivo*⁵. Furthermore, there is evidence that the rate-limiting step which leads to the accumulation of the precursor particles *in vivo* does not involve spontaneous conformational change of these particles⁶. Although all the information, therefore, required to construct a ribosome must be present in its components, it seems that cells use an additional mechanism to facilitate their assembly.

We report that reconstitution of 30S particles occurs *in vitro* with a much lower energy of activation if the precursor form of 16S RNA (p16S)⁷ is used in place of the mature RNA (m16S), and if the RNA is complemented with ribosomal proteins (r-proteins) extracted from nascent rather than from pre-existing ribosomes. We suggest that to facilitate ribosome assembly bacterial cells use a morphopoietic factor which recycles between precursor particles and completed ribosomes in concomitance with the maturation of rRNA.

The ionic conditions of Traub and Nomura⁵ were used both to dissociate and to reassociate ribonucleoprotein particles. A mixture of p16S and m16S RNA, differentially labelled with ³H- and ¹⁴C-uracil and obtained from particles sedimenting on a sucrose gradient at 25-30S, was incubated with increasing amounts of r-proteins extracted from total ribosomes. Incubation was carried out for various times both at 0°C and at 40°C to determine the temperature dependence of the reconstitution of 30S particles.

To obtain an efficient incorporation of rRNA into 30S particles a twofold excess of 30S proteins had to be used. In these conditions reconstitution of 30S particles occurred only at 40°C (Fig. 1a). At 0°C both p16S and m16S RNA formed particles sedimenting at 20-22S (Fig. 1b). Thus in the standard conditions of Traub and Nomura p16S RNA is no more efficient than m16S RNA in forming ribosomal subunits, confirming a recent observation⁸.

When the r-proteins/rRNA ratio in the incubation mixture was increased to a value greater than 3, some p16S RNA was incorporated into particles sedimenting at 30S even at 0°C. The amount of ³H-labelled 30S formed at 0°C did not increase with the time of incubation. Incorporation was the same when the mixture was analysed in sucrose gradients after an incubation of 2, 20 or 60 min, although it did increase with the amount of r-proteins added. At a protein/RNA ratio of 10 to 12, all p16S RNA was incorporated into 30S particles at 0°C and m16S RNA formed particles sedimenting at about 25-26S.

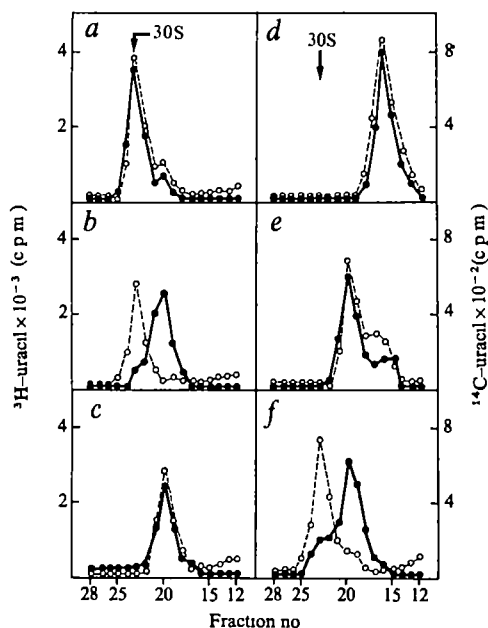


Fig 1 Reconstitution of 30S particles from p16S and m16S RNA in the presence of different amounts of r-proteins. ^3H -labelled p16S and ^{14}C -labelled m16S RNA were obtained from *E. coli* D10 cells growing in MS9 medium supplemented with 0.2% glucose and 0.5% casamino acids¹⁰. The culture (20 ml) was labelled with $1 \mu\text{Ci } ^{14}\text{C}$ -uracil (\bullet) for two generations and with $150 \mu\text{Ci } ^3\text{H}$ -uracil (\circ) for the last 2 min of growth. Cells were extracted at an A_{420} of 1.0 by pouring on to crushed ice, collected and lysed as previously described¹⁰. The lysate (1 ml in 10^{-2} M MgCl_2) was centrifuged on a sucrose gradient in 10^{-4} M MgCl_2 for 7.5 h in an SB238 International rotor at 40,000 r.p.m. The 30S peak was localised by counting an aliquot of each gradient fraction. Fractions containing material sedimenting from 25 to 30S were pooled and mixed with an equal volume of 8 M urea, 6 M LiCl. After 24 h the RNA was pelleted by centrifugation and redissolved in 0.3 M KCl, 20 mM Tris-HCl (pH 7.6), 2 mM MgCl_2 , 6 mM β mercaptoethanol and dialysed against the same buffer. An aliquot of RNA was precipitated with ethanol, redissolved in 1% sodium dodecyl sulphate and analysed using polyacrylamide gel electrophoresis as described previously⁹. The majority (90%) of the ^3H -labelled RNA (p16S) moved as a single sharp peak 6–8% slower than the ^{14}C -labelled RNA (m16S). Ribosomal proteins were extracted by the same LiCl-urea treatment both from total ribosomes and from native subunits and 70S particles. In the first case a crude extract of alumina ground cells was layered on top of a 5 ml sucrose gradient in 10^{-2} M MgCl_2 , and centrifuged long enough to pellet all ribosomal particles including native subunits and their precursors. In the second case, native subunits and 70S ribosomes were displayed on a regular sucrose gradient in 10^{-2} M MgCl_2 , localised by the A_{260} absorbance and collected by pelleting from the corresponding fractions. The pellets were resuspended in 4 M urea, 3 M LiCl, and 24 h later RNA was removed by centrifugation and supernatants were dialysed against 1 M KCl, 20 mM Tris-HCl (pH 7.6), 20 mM MgCl_2 , 6 mM β mercaptoethanol. To prepare the reconstitution mixture a sample of the RNA preparation was mixed at 0°C with the volume of protein preparation required to obtain the desired r-protein/rRNA ratio. KCl concentration was then reduced to a final value of 0.3 M by adding the appropriate volume of a buffer containing 20 mM Tris-HCl (pH 7.6), 20 mM MgCl_2 and 6 mM β mercaptoethanol. The relative amounts of RNA and protein were calculated from the volume of culture from which they had been obtained. After incubation at the temperature and for the time indicated below, the reconstitution mixtures were centrifuged on sucrose gradients in 10^{-3} M KCl, 10^{-2} M Tris-HCl (pH 7.6) for 7 h at 40,000 r.p.m. to display 30S particles and reconstitution intermediates formed. *a*, Protein from total ribosomes added in a ratio of 2.1 with RNA, incubated at 40°C for 20 min, *b*, as *a* but incubated at 0°C for 60 min, *c*, protein from total ribosomes added in a ratio of 12.1, incubated at 0°C for 5 min, *d*, protein from 70S ribosomes in a ratio of 4.1, incubated at 0°C for 5 min, *e*, protein from 70S ribosomes in a ratio of 30.1, incubated at 0°C for 5 min, *f*, protein from native subunits in a ratio of 4.1, incubated at 0°C for 5 min. The direction of sedimentation was from right to left.

(Fig 1c) ^{14}C -labelled 30S particles containing m16S RNA appeared at 0°C only at a protein/RNA ratio of about 30.

^3H -labelled 30S particles formed *in vitro* at 0°C starting from p16S RNA were capable of responding to T4 specific messenger RNA by entering polyribosomes when incubated in conditions for protein synthesis (data not shown, for detail on the assay see refs 6 and 9). This response was observed even when the assay was run at a temperature as low as 18°C to avoid the exposure of reconstituted particles to 37°C . On the basis of their ability to respond to T4 messenger RNA together with their sedimentation properties, therefore, ^3H labelled reconstituted particles resembled native 30S subunit and differed from the ^{14}C -labelled intermediary complex formed in the same reconstitution mixture starting from m16S RNA.

p16S RNA can thus form 30S particles in the cold more efficiently than m16S RNA. Several possibilities may be advanced to suggest why this does not happen in the presence of stoichiometric amounts of r-proteins but only in the presence of a large excess. Some ribosomal proteins could be partially inactivated during the extraction procedure. At 40°C , however, a protein/RNA ratio of 2–3 was sufficient to convert all rRNA into 30S particles, indicating that the putative inactivation was reversed by heating. Preheating r-proteins at 40°C before mixing with the RNA, however, did not relieve the high ratio requirement for the reconstitution of 30S at 0°C . Second at 0°C the 20–22S complex formed could have a much lower affinity for the missing proteins than at 40°C . When the RNA concentration was also increased twenty-five to thirtyfold that is, when stoichiometric amounts of RNA and protein were incubated at a very high concentration, no 30S formation occurred.

Third, a factor present only on a minor fraction of ribosome could lower the energy of activation of 30S assembly, allowing it to occur rapidly at 0°C . To test this hypothesis, we extracted proteins from 70S ribosomes and from native ribosomal subunits separated by sedimentation on a sucrose gradient. The preparation of ribosomal subunits included ribosomal precursor particles. As shown in Fig 1d and e, proteins extracted from 70S ribosomes did not sustain the formation of 30S particles in the cold even at a protein/RNA ratio of 30. Complete incorporation of p16S RNA into 30S particles was instead obtained with proteins extracted from native subunits at a ratio of 4 (Fig 1f).

The assembly of 30S particles *in vitro* may therefore be facilitated by a factor which can be extracted with the proteins from newly formed or precursor ribosomal particles, but is absent from most mature ribosomes. The factor acts preferentially on the precursor form of 16S RNA and, in the conventional reconstitution system used here, does not seem to function catalytically, but to be used up by the assembly reaction.

We propose that in the cell a factor exists which has the role of lowering the energy of activation required to convert precursor particles into 30S subunits. Because of its higher affinity for p16S than for m16S RNA, the factor would be released from completed ribosomes only after rRNA is matured. rRNA maturation, on the other hand, probably occurs in polyribosomes (ref 9 and G.M., E.T., C.P. and F.A., unpublished). Thus the recycling of the morphopoietic factor, and therefore the completion of nascent ribosomes, would be subordinated to the entry of previously formed particles into the pool of functioning ribosomes.

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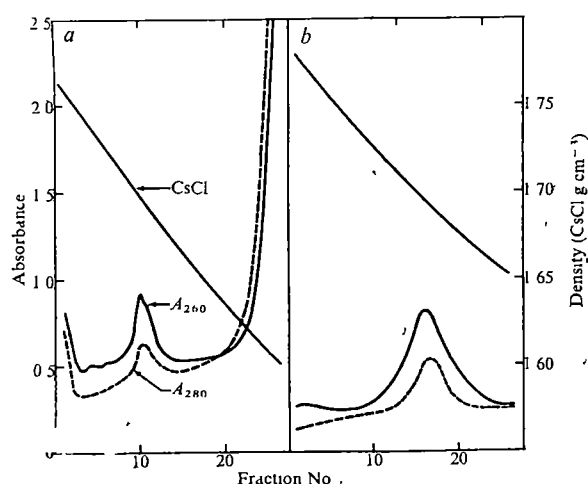
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Hybridisation of Dane particle DNA with the free plasma DNA of hepatitis carriers

PLASMA of hepatitis B patients and antigen carriers contains a high concentration of spherical and filamentous particles (HBsAg), about 20 nm in diameter and without evidence of nucleic acid. There are also a relatively few spherical particles, 42 nm in diameter with a 28 nm core, first described by Dane *et al*¹. Dane particles contain a unique deoxyribonucleic acid² and a DNA polymerase³ capable of synthesising DNA so they are considered to be a leading candidate for a human hepatitis B virus. Double stranded DNA of unique base composition has been isolated from the naked intranuclear particle of hepatocytes of human hepatitis liver⁵. The large quantity of HBsAg, resembling defective virus, in the plasma of carriers prompted us to investigate a possibility that there may exist free or 'unassembled' DNA corresponding to the empty particles in these plasmas. The presence of both free DNA and empty particles could result from faulty assembly of a virus. We report here the presence of free DNA in HBsAg positive plasma and this free DNA showed homology with radiolabelled Dane particle DNA by molecular hybridisation.

Plasmas from commercial donors were screened by radioimmunoassays for HBsAg according to Ling *et al*⁵. In a typical procedure for DNA isolation, about 50 ml of plasma were dialysed against Tris buffer (0.01 M Tris-HCl, pH 8.25, 0.1 M NaCl) containing 0.01% diethylpyrocarbonate. Solid caesium chloride was added to the dialysed plasma to an average density of about 1.65, and the solution was then centrifuged in six buckets of a Spinco SW41 rotor at 35,000 r.p.m. for 67 h at 22°C. Because of the large amount of serum protein present in the sample, absorbance readings of the collected fractions were high and generally did not reveal separation of components. Occasionally, however, a small peak of absorbance at 260 nm appeared above the high background around a density of 1.7.

Fig 1 a, Isopycnic centrifugation of plasma in CsCl, b, the second CsCl centrifugation of the combined fractions around the density of 1.7 in a



(Fig 1a) Fractions around this density, regardless of the presence of an absorbance peak or not, were combined and dialysed against the same buffer. One or two recentrifugations of the sample in CsCl revealed DNA, if present, at a density of 1.698. The absorbance profile of a final centrifugation from a DNA-positive sample is shown in Fig 1b. The DNA showed a density of 1.423 in Cs_2SO_4 , $S_{20,w}$ values ranging from 10 to 22, and a T_m of 72°C in phosphate buffer (0.01 M, pH 7.15, 1 mM EDTA). The DNA appeared to be composed of linear double stranded molecules of about 2–3 μm . Details of the characterisation of this DNA will be described elsewhere.

Synthesis and isolation of 3H -thymidine labelled DNA from Dane particles were carried out as previously described³. Because of the minute quantity of DNA recovered from Dane particles, specific activities of the radiolabelled DNA could not be estimated. Hybridisation of the radioactive DNA and the plasma DNA was done according to a published method⁶ with the following modifications. About 2,500 c.p.m. of 3H -thymidine labelled DNA from Dane particles, and the non-radioactive

Table 1 Hybridisation between free DNA of plasma and 3H -DNA of Dane particles

Plasma	No of samples	No of DNA-positive sample	No of DNA-positive sample hybridised	No of positive hybridisation
HBsAg positive	21	21*	5	5†
HBsAg negative	21	2	2	2

* In a DNA-positive sample, A_{260} readings of the collected fractions from a 50 ml plasma were at least 0.01 above the background after the second CsCl centrifugation.

† In a positive hybridisation 1 μg of DNA protected more than 50% of the radio labelled Dane particle DNA from S1 nuclease digestion. Details of hybridisation conditions are described in the text.

plasma DNA were denatured, separately, with NaOH at pH 13 for 60 min at 37°C. Both DNA samples were then neutralised with HCl and incubated together in 1 \times SSC in a total volume of 130 μl for 4 h at 68°C. The quantity of cold DNA varied from 0.1 to 1.0 μg , estimated from spectrophotometric absorption where 24 A_{260} units were equivalent to 1 mg. After incubation, unhybridised, single-stranded DNA was digested with 1,000 units of S1 nuclease (Seikagaku Kogyo, Japan) for 2 h at 37°C. Calf thymus DNA (50 μg) was then added as a carrier and the reaction mixture was pipetted on 3 mm paper for washings in TCA according to Mans and Novelli⁷. Radioactivity was determined in a Beckman scintillation spectrometer. Negative controls in which cold DNA was omitted from hybridisation, served as the background of self-annealing which averaged about 10–15% of the input radioactivity. The value of 100% hybridisation was provided from reaction mixtures treated identically except that no S1 nuclease was used. DNA from human diploid cells (WI 38) and human liver tissue was isolated according to a published procedure⁸. Salmon sperm DNA and calf thymus DNA were purchased from the Sigma Chemical Company. These four DNA preparations were sonicated to give sizes comparable to plasma DNA with a $S_{20,w}$ range of 10–25.

It was evident from the results of hybridisation (Fig 2) that the DNA isolated from HBsAg positive plasma by isopycnic banding in CsCl possessed homology with the radioactive Dane particle DNA. Similar results in hybridisation were obtained with DNA after further purification by phenol extraction and alcohol precipitation. DNA from human liver, WI 38 cells, salmon sperm and calf thymus showed no evidences of homology. In the experimental conditions used, the C_0t_1 of the plasma DNA was estimated to be about $9 \times 10^{-2} \text{ mol s}^{-1}$. This value was considerably higher than that of a theoretical value of hybridisation (5×10^{-3}) between Dane particle DNA having a molecular weight of 1.6×10^6 with complexity of 2,600 nucleotide pairs¹⁰. This difference in observed and calculated C_0t_1 values could be due to two reasons. First, the hybridisation studies

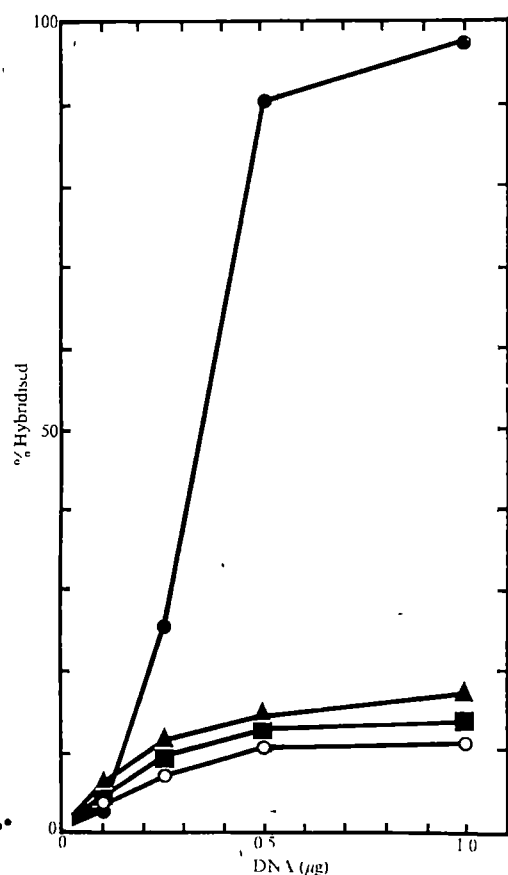


Fig 2 Molecular hybridisation of plasma and heterologous DNA with ^3H -DNA of Dane particles (●), HBsAg plasma DNA, ▲, salmon sperm DNA, ■, WI 38 DNA, ○, human liver DNA

were conducted with molecules ($S_{20,w}$ of 10 to 22) much larger than small fragments of 400 to 500 nucleotides used to calculate the complexity of DNA from C_0t_1 values⁹. Thus the slower kinetics of reassociation with larger molecules would increase the C_0t_1 value¹⁰. Second, DNA not related to Dane particles, if present in the plasma DNA, would increase C_0t_1 . Further studies will be necessary to establish the full extent of homology between the DNA from plasma and the DNA produced by Dane particle polymerase.

Having found free DNA in a few plasmas from HBsAg carriers, we undertook a survey of 42 plasmas with regard to the presence or absence of free DNA and the ability of the DNA, if present, to hybridise with Dane particle DNA. The results of the study are shown in Table 1. In all of the 21 HBsAg positive plasmas examined, we found detectable amounts of free DNA with a recovered quantity equivalent to about 0.1–1 $\mu\text{g ml}^{-1}$ of plasma. Five of these twenty-one DNA preparations were selected randomly and were found hybridisable to ^3H -DNA of Dane particles. In the group of HBsAg negative samples we found the majority of samples (19/21) negative in free DNA in that no detectable absorbancy was found upon purifying the 1.7 density regions. Two of the samples, however, showed the presence of free DNA, and this DNA showed positive hybridisations with Dane particle DNA. The significance of this observation is not clear at this time, as we do not know whether free DNA in plasma is of host or viral origin. Further studies of these two plasmas are underway to assess fully the possible presence of HBsAg, undetectable with the methods employed.

The average length of the plasma DNA in electron micrographs was estimated to be 2–3 μm , equivalent to a molecular weight of 4×10^6 – 5×10^6 . In some plasmas, the DNA concentration was the order of 1 $\mu\text{g ml}^{-1}$, equivalent to 10^{10} molecules ml^{-1} . By comparison, a HBsAg concentration of 100 $\mu\text{g ml}^{-1}$ in the plasma could give 3×10^{13} particles ml^{-1} , based on a

molecular weight of 2×10^6 daltons per particle. The length of DNA was longer than that of the circular genome of Dane particle DNA (0.78 μm). We have observed rolling circles in electron micrographs of the DNA isolated from Dane particles actively synthesising DNA (ref. 3). These rolling circles¹¹ with different lengths of linear segments would represent progeny DNA in polymeric forms. Although we found considerable homology between the DNA in plasma and in Dane particles, it is yet to be proven that the plasma DNA contains homology equivalent to one or more copies of the Dane particle genome.

The Dane particle is a leading candidate for the hepatitis B infectious agent, based on the findings that they contain a unique DNA primed with a DNA polymerase and are found in infectious plasmas of HBsAg carriers. In spite of the routine screening of blood donors with sensitive HBsAg immunological procedures, only about half of the cases of hepatitis B associated with blood transfusion seem to be related to the presence of HBsAg or antibody in donor blood, as revealed by these methods. The findings in this report on the presence of free DNA having homology to the DNA of Dane particles suggests the possibility of using molecular hybridisation in conjunction with immunological techniques for assessing potential infectivity of blood plasmas.

We thank Dr T. Kakefuda for electron microscopic studies of DNA, Dr W. Holleman for analytical centrifugation studies and W. Schulze for technical assistance.

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Errata

IN the article "Flow near an oscillating cylinder in dilute viscoelastic fluid" by C. Chang and W. R. Schowalter (*Nature*, **252**, 686, 1974), Figs 2b and 3b should be interchanged.

IN the article "Ambisonic reproduction of directionality in surround-sound systems" by P. B. Fellgett (*Nature*, **252**, 534, 1974) the following corrections are necessary: Page 537 line 6, for π read 2π , line 9, for hyper-spheres read elliptic spaces, in ref. 3 for 189 read 1892 (date), in Fig 2c the right-hand side of the CBS pan locus should be solid not dashed, to show that it is at the front (not the rear) of the sphere.

IN the article by A. I. Cunningham (*Nature*, **252**, 749, 1974) the title should read "Large numbers of cells in normal mice produce antibody against components of isologous erythrocytes" and not as printed.

reviews

In January 1972 a study group of psychologists and ethologists met in London to discuss the growth of competence, under the aegis of the Developmental Sciences Trust and the Ciba Foundation. This book represents the fruits of their discussion; it comprises 16 papers presented either at the symposium or commissioned during its aftermath for inclusion in the published proceedings.

Most of the original papers have been revised in the light of comments made at the conference, and the editors have written introductory and concluding chapters. It is unusual, however, for such manoeuvres to transform a collection of individual papers on a common theme into a plausible book in which an identifiable argument is developed, and it must be said that *The Growth of Competence* has several of the characteristic flaws of the standard conference book. The most serious of these may be anticipated: the word 'competence' has such wide vernacular use that it comes as no surprise to discover between the book's covers almost as many definitions of its central concept as there are contributors. And even though this state of affairs may arise from the nature of the concept rather than from lexicographical laxness on the part of the authors, its

Competence in many forms

John N. Nicholson

The Growth of Competence. (Proceedings of a DST Study Group.) Edited by Kevin Connolly and Jerome Bruner. Pp. xii+327. (Academic: London and New York, September 1974.) £6.80; \$17.50.

effect on the book may be imagined. There are, moreover, a number of chapters which can with charity be described as lightweight. They neither include material interesting in its own right nor contribute to the argument which, in spite of the handicap mentioned above, is developed in the book; they are presumably included in deference to the book's function as a document of record.

These shortcomings are particularly regrettable in view of the book's praiseworthy intentions and the admirable quality of some of the contributions. It sets out to describe competence; to place it in an evolutionary context (a wide-ranging chapter by Bruner); to exemplify its development in a number of areas, including infants'

relationships with their mothers and their peers, skills (a detailed description of Elliott and Connolly's pioneering analysis of manual skills in normal and cerebral palsied children), language acquisition and personality; and to point out the practical applications of the experiments discussed and theoretical issues raised.

The book's central argument is carried forward by those contributors who follow Bruner in likening the growth of competence to the development of skills, which he regards as an hierarchically organised repertoire of sub-routines. This analogy is pushed furthest by Susan Carey in a provocative chapter on the development of cognitive competence, based on her penetrating reanalysis of the conservation problem. But in the end the book cannot escape the limitations of its genre. Most of the experimental work has been described elsewhere; it is unlikely therefore to become mandatory reading amongst developmental psychologists. Educationalists may welcome the discussion of the efficacy of schools as socialising agents in contemporary society, but they will find little cheer in the editors' pessimistic view of the contribution psychologists have thus far been able to make towards the solution of such problems. □

The subject is fascinating. How is it that the principal area of technological rivalry between the two super-powers could within two years become the main field of cooperation in advanced projects? What shifts in foreign policy, in the scientific climate, even in personalities, mediated so radical a 'thaw'? Alas, one is not going to get more than a few hints from this book.

Was the untimely death of Dr Hugh Dryden, NASA's chief negotiator during the difficult early 1960s a significant setback? Did the 1964 Committee on Space Research (COSPAR) agreement on planetary contamination standards mark the first visible breakthrough of reason over rivalry (as was then widely reported)? There is no assessment of the first point and the second is not mentioned.

Disappointment with the book at first prompts the judgement that it is quite irrelevant. It is not; it is just biased. The book has apparently been developed through a series of studies by a team of authors drawing on a privileged

archive at the Center for Advanced International Studies of the University of Miami (CIAS). It presents a digest of official, documentary evidence and American reportage (which are given equal validity) within the matrix of the authors' own commentary.

The whole thing is set rolling in a predetermined direction with a 28-page

Detente in orbit

Angela Croome

U.S.-Soviet Cooperation in Space. By Dodd L. Harvey and Linda C. Ciccoritti. Pp. 350. (University of Miami: Florida, 1974.) \$8.95, paper; \$12.95, boards.

foreword by the United States Ambassador to Moscow in the mid-1960s, Foy D. Kohler, who is now luminary of CIAS. He discounts any possibility of an independent role or influence from Soviet scientists. He sees the entire pattern of cooperation as determined by Soviet political pressures. He may be right but his own professional

allegiance reduces any conviction and the material adduced suggests that there has been some careful selection to support his thesis. Nor does he acknowledge the equally overriding influence of American political postures on the turn of events during the 15-year period leading up to the 1972 Nixon-Brezhnev Agreement (the space cooperation programme was, significantly, a part of the overall detente agreement).

It is useful to have chapter and verse of the Dean Rusk papers (1962) which concluded that space had no military value for the United States and which suggested that the Soviet Union should appreciate this before adopting counter measures to a non-existent threat. That was a turning point.

The volume's 100-page bibliography would be invaluable if it could be consulted independently of the text. As it is, this 'analysis' is journalistic in approach but does not have the convenience of having been written by journalists. □

Magnetic models

Experiments on Simple Magnetic Model Systems: A Survey of their Experimental Status in the Light of Current Theories. (Taylor and Francis Monographs on Physics.) By L. J. de Jongh and A. R. Miedema. Pp. 269. (Taylor and Francis: London, August 1974.) £4.00.

Forty years elapsed between the discovery of superconductivity and the advent of a theoretical model describing the effect. A much greater period had passed at around the turn of the century when Weiss introduced the first theoretical model to account for another cooperative phenomenon, ferromagnetism. Since that time, increasingly complex and subtle models have been developed to allow more accurate calculations of thermodynamic quantities near phase transitions. The simplest models, restricted to one or two-dimensional crystal lattices, enable exact calculations and may seem to provide merely a playground for the theoretician. But those models now have, in fact, a relevance to the real world. Advancing technology has facilitated the identification and preparation of single crystals containing chains or layers of paramagnetic ions, isolated by distances of the order of tens of Angstroms, which, therefore, have a magnetic quasidimensionality of one or two.

A large part of this book is devoted to a review of the measured properties of such compounds and, although it is not easy reading, it provides an invaluable, and what must be an almost exhaustive, reference catalogue of these materials—the index to substances contains approximately 200 entries. The fitting of theory to experiment by judicious choice of the exchange constant is impressive in many cases.

There remain, however, some areas in which the available experimental analogues do not yet approximate closely enough to the models to confirm some theoretical predictions. An example of this is the two dimensional Heisenberg model, in which long-range order must be absent at finite temperatures: the expansion approach of the high temperature series suggests that the susceptibility will diverge at a finite temperature. The problem is resolved by identifying this temperature not with the normal phase transition but as a transition (the Stanley-Kaplan transition) to a state of infinite initial susceptibility with zero spontaneous magnetisation. Real materials deviate from the ideal because of the presence of anisotropy from single ion or dipolar contributions. Competing anisotropies can, however, be made to cancel, al-

though usually at a single temperature only. The best approach, therefore, seems to be to use a series of compounds in which the varying degree of deviation can be determined, then to extrapolate to the ideal. The authors believe that the evidence so obtained suggests that Stanley-Kaplan temperatures may exist.

In a final section a number of special topics are considered, including neutron diffraction and spin-wave theory.

This book, a reprint of *Advances in Physics*, 23, contains an elegant and concise review of the properties of the theoretical models of magnetic systems, and a comprehensive body of experimental data relevant to those models. As such, it is a useful addition to the library of the research worker in solid state magnetism.

W. O'Reilly

Insect behaviour

Experimental Analysis of Insect Behaviour. Edited by L. Barton Browne. Pp. viii+366. (Springer: Heidelberg, Berlin, New York, 1974.) \$15.40.

This volume originates from a symposium entitled "Experimental Analysis of Insect Behaviour" which formed part of the 14th International Congress of Entomology held in Canberra in 1972. The 25 contributions to the book include several not actually delivered at the symposium and some that are modified versions of the original papers. Most are written in the form of reviews, some of which are speculative,

emphasising particular areas of research, and some of which are of a more general nature.

Appropriately, because he addressed the closing plenary session of the Congress on "The emergence of behaviour", J. S. Kennedy's paper—"Changes of responsiveness in the patterning of behavioural sequences"—sets the tone of the volume by summarising his interpretation of flight and settling responses in *Aphis fabae*. The final paper, by Dingle, also deals with insect flight in the context of "The experimental analysis of migration and life history strategies in insects" and it is refreshing and encouraging to find many ecological concepts aired in such physiological company. Topics included in the intervening 23 papers include reproductive behaviour, feeding, several aspects of the insect nervous system and neural mechanisms, and hormone-mediated behaviour. Many of the papers are useful appraisals of recent work and concepts in the experimental analysis of insect behaviour, and it is convenient to have them so readily available.

Unfortunately, the editor has made little attempt to arrange the papers to present and develop an underlying theme, or to summarise the forward-looking approach that the symposium was, presumably, convened to stimulate. Indeed, the volume—reproduced from typescript—can only be considered as a 'book' in so far as it is bound between boards.

T. Lewis



Burial tombs of Tasmanian aborigines. From *Biogeography and Ecology in Tasmania*. (Monographiae Biologicae, vol. 25.) By W. D. Williams. Pp. 498+122 figs. (Junk: The Hague, 1974.) Dfl 140.

Physical Chemistry: An Advanced Treatise. Vol. VIA: *Kinetics of Gas Reactions*. Edited by William Jost. Pp. xx+507. (Academic: New York and London, November 1974.) \$43.00; £20.25.

THE editors of this series note in the Foreword that, because of the tremendous expansion in the development of techniques and principles of physical chemistry in recent years, most physical chemists "find it difficult to maintain an understanding of the entire field". This surely understates the problem, and I must admit, albeit sadly, that I find this difficulty even when restricted to my own chosen field of physical chemistry—kinetics—let alone the "entire field".

An advanced treatise should assume that the reader has at least a first degree level of knowledge, and this is certainly true of this volume. Indeed in some cases rather more is assumed of the reader.

The first chapter, on formal kinetics, by W. Jost makes an excellent start with a concise but clear survey of classical reaction kinetics, and leads on to a consideration of steady states, stability and, finally such topics as oscillating reactions; these latter topics have attracted much attention in the past decade. The next, short chapter, by C. F. Curtiss—A Survey of Kinetic Theory—is, in my opinion, too limited in scope and is at a level which surely assumes too much of a graduate student. It does not fit in happily with the rest of the volume. In the third chapter by H. Eyring and S. H. Lin, on potential energy surfaces, an account of the valence bond method is given and then surfaces for $H+H_2$, $Cl+Cl_2$ and $K+NaCl$ are considered in some detail. A final section on orbital symmetry in reaction kinetics does not blend well with the rest of the chapter and deserves a chapter of its own.

Chapter 4 is again short—E. E. Nikitin on the Theory of Energy Transfer in Molecular Collisions. The translation, though clear, is a little stilted and the treatment is too condensed; few readers will find it an easy introduction to this important topic. The final chapters constitute more than half this volume. Chapter 5 by J. P. Toennies is on molecular beam scattering experiments and considers elastic, inelastic and reactive collisions. This is a field that is progressing rapidly and this chapter presents a balanced and clear account of it. Chapter 6 by J. C. Polanyi and J. L. Schreiber on the dynamics of bimolecular reactions must, of necessity, cover some of the same ground as the earlier chapters. Such duplication as there is, however, is not serious, and the viewpoint is

somewhat different. Again, this chapter presents a clear account which most graduate students interested in kinetics will find valuable.

The chapter headings to the companion volume (VIB) on gas kinetics suggest that the two will fit together well. It is, however, sad to note how many important areas of gas kinetics will not be covered at all.

This book can be recommended for libraries, but at £20.25 it is hardly cheap and is unlikely to find itself on the shelves of all those 'physical chemists' for whom it was, no doubt, intended.

H. M. Frey

Gas works

Theory of Elementary Atomic and Molecular Processes in Gases. By E. E. Nikitin. Translated by M. J. Kearsley. Pp. xiii+472. (Clarendon: Oxford; Oxford University Press: London, August 1974.) £14.75.

THE author of this book is one of the world's leading authorities on the theory of rate processes, who has extensive and detailed research experience in many branches of the subject. This translation from the Russian version originally published in 1970 is, therefore, more than welcome. The emphasis rests on the theory of processes relevant to the chemical reactions discussed. The main body of the book makes reference to the literature published before the end of 1966, with an added appendix that includes brief reference to developments in the two or three years following that date.

Professor Nikitin presents a very wide, well integrated view of the subject as a whole, and thus the material in the various chapters of the book is well organised, with chapters following one another in a smooth fashion. The first chapter on the transition state method is presented in a very formal manner, going far beyond the statistical mechanical treatment normally found in standard books on kinetics. The following chapter, dealing with the transfer of translational motion to other forms of energy, especially to vibration, for which there is a large body of experimental data, and also to electronic energy in both atoms and diatomic molecules, is extensive and highly authoritative.

The chapter formally entitled "Unimolecular Reactions" may seem to be relatively limited, considering the scope of the subject, but it is a tight and formal approach. It follows well the chapter concerned with vibrational energy transfer and includes a section on surface crossing and "non-adiabatic

transitions". With that chapter one should also consider the following detailed treatment of the statistical theory of reactions. Similarly, the presentation of the diffusion theory of reactions, which regards chemical reactions as diffusion processes of representative points in phase space from regions corresponding to reactants to regions corresponding to products, is also highly relevant to the considerations of unimolecular reactions given in earlier chapters.

The dissociation of diatomic molecules and the reverse process of atomic recombination are dealt with more in terms of the types of results obtained from different theories than in terms of detailed developments of the leading theories. The appeal to experiment is very limited but the chapter is rich in the physical principles at issue in such processes. The basis of semi-empirical methods for bimolecular reactions is presented with particular clarity, and leads in due course to the more recent, *ab initio* calculations of Bruner and Conroy for the H_2 system.

Direct reactions and the dynamics of exchange reactions, of particular relevance to processes studied in molecular beams, are also dealt with fully. The appendix, clearly written after the main body of the book, is a useful brief résumé of the objectives of theories of chemical reactions. As the book is designed for post graduate chemists and physicists, the appendix could usefully be read first, especially as there is no general introduction.

Since the completion of the book, there has been a major development in the experimental study of many fundamental aspects of atomic reactions. Work on the collisional behaviour of atoms in specific electronic states, and various studies aimed towards the development of a framework within which context the relationship between electronic structure and atomic reactivity can be understood, comprise much of this development. These areas of research occupy little space, however, and it is to be hoped that the next edition will contain much more of that type of work, including Professor Nikitin's own theoretical researches.

For research workers concerned either with the theory or experiment of fundamental rate processes in gases, access to this book is barely a matter of choice but rather of basic necessity. Occasionally, the price of a book must be judged not only in relation to its size but to the quality of its content. This is such a book; it is hard to imagine a library without it, and it is to be hoped that research workers will consider that a classic work of this kind merits a place on their personal bookshelves.

D. Husain

obituary

Francis J. Weiss, the biochemist and economist concerned with developing food resources, died on January 21, at the age of 76.

Dr Weiss was born in Vienna, receiving doctorates in chemistry and economic statistics at the University there. He worked in the biochemistry institute of the University of Vienna until the Nazis occupied Austria in 1938, at which time he left for England. A year later, he went to the United States, where he was a consultant to the Board of Economic Warfare and the Sugar Research Foundation. In the fifties, he was a consultant on the staff of Senator Hubert H. Humphrey, studying chemical agriculture and new methods of processing grains. Later, he was a consultant to the International Cooperation Administration and the Department of Commerce. From 1959 to 1969, he was a specialist in the science and technology division of the Library of Congress.

Vladimir Aleksandrovich Fock, academician, and one of the leading theoretical physicists in the Soviet Union, died on December 27, 1974, five days after his 76th birthday.

Fock, who graduated from the University of Petrograd (now Lenin-

grad) in 1922, throughout his working life remained connected with this University, teaching and researching there. He also at various times worked at the State Institute of Optics, the Leningrad Institute of Physics and Technology, and the Institute of Physics of the Soviet Academy of Sciences. In 1932 he was elected a Corresponding Member, and in 1939 a full member of the Soviet Academy of Sciences. Fock's basic research was concerned with quantum mechanics, quantum electrodynamics, electromagnetic diffraction and radiowave propagation, the general theory of relativity, mathematics, and mathematical physics, in all of which fields he established new basic concepts. He is particularly associated with the establishment of the scalar relativistic wave equation for a particle with no spin in an electromagnetic field, which he derived independently of similar work by the Swedish physicist O. Klein (the 'Klein-Fock equation'). His name is also associated with spaces of an increasing number of dimensions ('Fock spaces'), which he used to obtain a quantum description of systems with a variable number of Bose particles. For his services to Soviet science, Fock was awarded the Order of Lenin (four

times), a Stalin Prize (now State Prize) in 1946 and a Lenin Prize in 1960. He was also a member of a number of foreign and international academies and learned societies.

Matthew W. Stirling, the distinguished archaeologist, died on January 23, at the age of 78.

An anthropologist and archaeologist associated with the Smithsonian Institution for more than 40 yr, Dr Stirling travelled widely in Central and South America, discovering "America's oldest dated work"—a stone monument bearing a date equivalent to 291 BC. He has headed many important expeditions in the Americas and in Europe, but will be known primarily for his contributions to middle American anthropology and archaeology. Under the auspices of both the Smithsonian and the National Geographic Society, Dr Stirling led the team of archaeologists who uncovered the La Venta, or Olmec, civilisation dating back more than 1,000 yr. Dr Stirling was born in California and graduated from the University there in 1920. He was affiliated with the Smithsonian from 1921 until his retirement in 1958. He also served as head of its bureau of American Ethnology from 1928 to 1947.

announcements

International meetings

March 13-14, **Global Tectonics in Proterozoic Times**, London (Executive Secretary, Royal Society, 6 Carlton House Terrace, London SW1Y 5AG, UK).

April 20-23, **Cybernetics and Systems Research**, Vienna (Secretariat of the Austrian Society for Cybernetic Studies, Schottengasse 3, A-1010 Wien, Austria).

May 6, **Crop Protection**, Gent, Belgium (The Secretary, Faculty of Agricultural Sciences, State University, Coupure links, 533, B-9000 Gent, Belgium).

Reports and publications

Great Britain

Proceedings of the Royal Irish Academy. Vol. 75, Section A, No. 14: Shrinking and Boundedly Complete Bases of Projections. By J. J. M. Chadwick. Pp. 95-102. 38p. Vol. 74, Section B, No. 18: Biology of the Rudd Scardinius erythrophthalmus (L) in Irish Waters.

By M. Kennedy and P. Fitzmaurice. Pp. 245-304 + plates 7-10. £1.47. No. 19: Curare and Post-Mortem Changes in Skeletal Muscle of Pietrain Pigs. By J. V. McLoughlin. Pp. 305-312. 18p. No. 20: The Upper Devonian and Lower Carboniferous Stratigraphy of the Whitegate Area, Co. Cork. By I. A. MacCarthy. Pp. 513-330 + plate 11. 36p. No. 21: The Action of Acid on Bisthiosemicarbazones and the Formation of Some Novel Heterocycles. By R. S. McElhinney. Pp. 331-380. 76p. No. 22: The Relationship Between the Acid and Basic Rocks near Carlingford, Co. Louth. By S. De and A. B. Poole. Pp. 381-402 + plate 12. 57p. No. 23: Identification of the Marine Species of the Genus *Vaucheria* in Ireland. By J. P. Cullinane. Pp. 403-410 + plate 13. 32p. No. 23: A Revision of the Caribbean Species in the Genera *Columnea* L. and *Alloplectus* Mart (Gesneriaceae). By B. Morley. Pp. 411-438. 37p. (Dublin: Royal Irish Academy, 1974.) [1912]

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nature

February 20, 1975

Did Dr Shah die in vain?

THREE years ago Dr V. H. Shah, a senior agronomist with the Indian Agricultural Research Institute (IARI), committed suicide in protest against the system prevailing in Indian agricultural research—and his was the third suicide of an agriculturalist in twelve years. His complaints, left in a letter to the director of IARI Dr Swaminathan, fell into two categories—first, that scientific results were being misrepresented to the extent that misleading and incorrect publicity was being disseminated; and second, that the career structure in agricultural research was being undermined by improper appointments. The failure of Dr Shah to be appointed to a professorship in agronomy was the immediate cause of his suicide, and in his letter he mentioned another appointment in agronomy which he also regarded as going to the wrong man.

Reaction was rapid. A committee of considerable eminence was established and reported in 1973. Since there were obviously more complaints in the air against the Indian Council for Agricultural Research (ICAR) than Dr Shah had spelt out, the committee investigated recruitment files and sent out questionnaires to a wide range of scientists.

To the accusation of scientific misrepresentation, the committee gave a mixed response. Dr Shah had named three specific cases, and in only one of the three did the committee go along with his accusation, although there were some sharp words on the way that advances in agricultural science were being publicised, and this criticism extended beyond the instances raised in the suicide note. The scientific issues have been rather extensively aired in *New Scientist* recently and need no further comment here.

The question of employment conditions and morale are, however, worth discussion. The committee unearthed much dissatisfaction and hard feeling within the agricultural community. This was directed at just about every aspect in their scientific life: appointments were said to be going to incompetents, library facilities were inadequate, there was too much power at headquarters, it was impossible to promote without there being a vacancy available (and competition for it) and equally impossible to get rid of incompetent workers. Perhaps most significantly, half the responses to the questionnaire on the question of what caused most difficulty in doing research blamed interference by supervisors.

The examining committee accepted the validity of much of this criticism. Indeed, the committee's own technical advisers themselves felt sufficiently strongly to write that the malaise which they had observed was not confined to ICAR, but "barring minor exceptions, pervaded the entire scientific and academic community of the

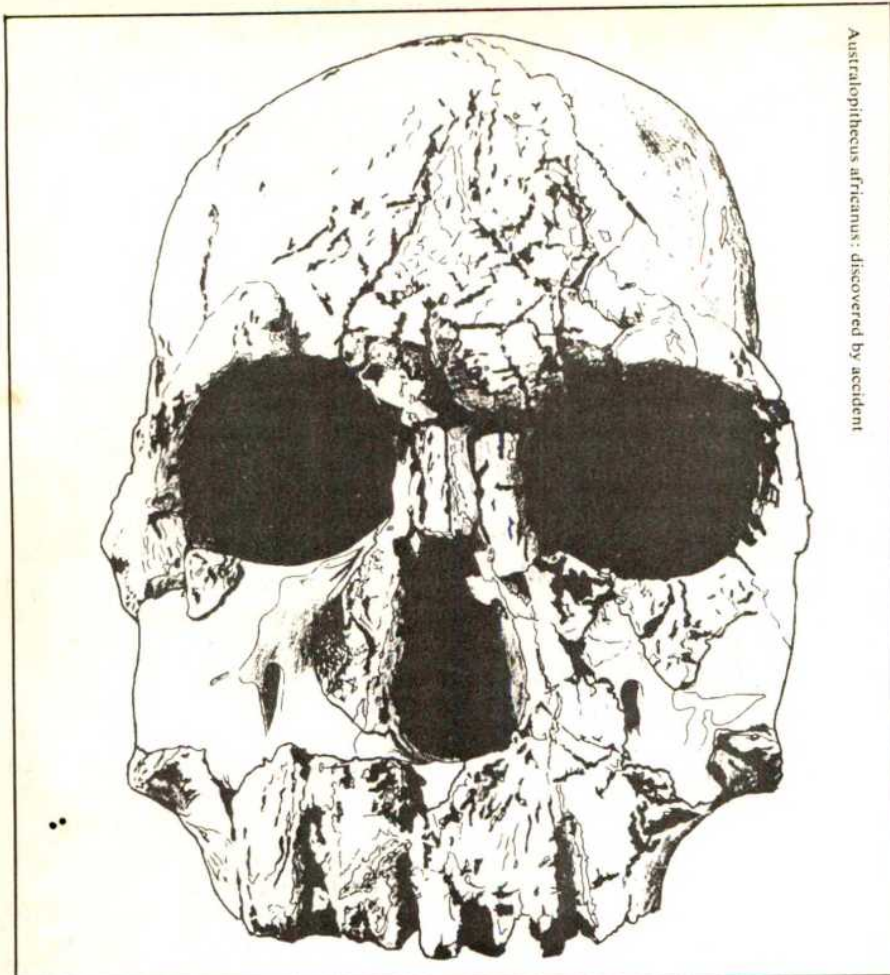
country. At root, it is greed for bureaucratic power and love of a comfortable life which afflicts this class. Juniors are intellectually as corrupt as seniors, and politicisation of academic and scientific life makes matters much worse."

Ironically the committee were not so impressed by two of the cases brought forward by Dr Shah himself. They found that the appointment which he had himself hoped to secure and which went to someone else was unobjectionable. And whilst they were critical of a second appointment made a few months earlier (Dr Shah had not applied for this post) there was considerable disagreement on whether a Ph.D in crop physiology plus ten years in agronomy was a suitable qualification for a post advertised as requiring a Ph.D in agronomy plus ten years' experience.

The almost obsessively narrow nature of this issue unfortunately provided a rather simple escape mechanism—better procedures in recruitment and promotion in ICAR. Unfortunate for two reasons—first because it is by no means clear that Indian science, already much stifled by its reverence for degrees, authority and seniority, needs even more rigid adherence to paper qualifications (a third of all who responded to the committee's questionnaire said that promotion should be based on seniority, without reference to merit). The best science doesn't always reveal itself to the best qualified. Second, because there is still no real sign that the Indian government sees more than administrative changes as necessary as a consequence of Dr Shah's suicide and this report. As the technical advisers bluntly put it, intellectual corruption exists at all levels; much more than changes in selection procedures are needed to eliminate it. The origins of this corruption are not difficult to find—they lie in the surfeit of bureaucracy, concentration of power at headquarters, a gross oversupply of graduates some of whom seem to acquire their degree only for matrimonial purposes, the shortage of money, the poor quality of technical help and the lack of diversity of opportunity within Indian science.

None of these problems is easily solved, but they are all potentially soluble. Mrs Gandhi, by being Minister for Science and Technology herself, is expressing a national faith that science and technology can still help India. She is in a powerful position to take bold steps to reduce mistrust and discontent amongst the scientific community, and she should take radical action.

We regret the poor quality of the paper on which last week's *Nature* was published. Continuity of paper supply has posed a continual problem for us, but we are doing everything possible to stabilise the situation.



Australopithecus africanus: discovered by accident

It is almost 50 years to the day that Nature published a report by Dr Raymond Dart describing the face, jaw and the brain case of a child belonging to what Dart described as "an extinct race of apes, intermediate between anthropoids and man". Nowadays, though chance and perspicacity still count in the search for hominid fossils, the scene has shifted from South to East Africa. The size of the cast has increased and both the extent of backstage support and the scale of the budget reflect the fact that hominid research has changed a good deal in the past half century. Bernard Wood reports.

THE child's skull which Dart called *Australopithecus africanus*, the southern ape, had been discovered by accident (*Nature*, 115, 195; 1925). It had been found in the rubble of a lime quarry at Taung, which is about 80 miles north of Kimberley in what was then Bechuanaland. It was fortunate that the remains of a fossil monkey had been recognised at the same quarry only a few months previously. The child's skull was in fact identified, and then passed on to Dart, by Professor Young, a geologist and one of those who had been alerted that the site was of potential interest.

The Taung child was not the first fossil hominid to be discovered; evidence had been found in the previous century at several sites in Europe and by Dubois in Indonesia. The Taung child was, however, much less 'man-like' than any of these other candidates for a relative or ancestor of man,

despite the fact that its young age tended to mask these non-human traits. Also it was the first specimen to be found in Africa, a continent that has since given up so much evidence to support Darwin's speculation that it was the cradle of mankind. In South Africa after Taung, Dart and Robert Broom, a noted palaeontologist, learnt of other cave sites in the dolomite of the Transvaal. Although no more hominid remains have been found at Taung, literally hundreds of specimens have been found at four other cave sites, and work has been resumed recently at three of these sites.

The other concentration of hominid fossil sites is in East Africa, and the circumstances could hardly be more different. The East African sites are all associated with the Rift Valley where hominid bones, and the evidence of hominid activity, are preserved in the silt of ancient lakes and streams, in-

stead of having been carried, washed or dropped into caves as in South Africa. The Rift Valley sites are only revealed to us now because earth movements have so distorted the landscape of several million years ago so that what were beds and shores of lakes are now thrust up and tilted so that they are exposed to erosion by water and wind.

An advantage of the location of these sites in sedimentary basins is that, with the help of palaeontologists and those skilled in palaeoenvironmental research, the life and landscape that the early hominids knew can be reconstructed. The intensity of diversification in contemporary animals and knowledge of the habitat are but two of the new dimensions that have been introduced into the interpretation of hominid remains. The stratigraphy of the sediments also enables the location of specific fossil finds within a large site to be placed in an order of occurrence. The tectonic activity that elevated and tilted the fossil sites was accompanied by volcanic eruptions, the products of which are suitable for isotope dating. These eruptions periodically covered the landscape and are now incorporated as layers of jam in the sedimentary sponge-cake. The dates of these strata provide bracketing ages for the fossils that lie in the intervening sediments. The history of the Earth's magnetic field can also be traced in the sediments which preserve the perturbations of the magnetic poles. When this information is combined with the results of the isotope dating techniques the East African site can be fitted into an absolute time scale enabling them to be placed in a historical context, one with another, and also with dateable sites elsewhere.

The problems of organising the team of research workers necessary for modern research into human origins, in inaccessible areas and often in a harsh climate, are probably no better illustrated than at East Rudolf in North Kenya.

This site, comprising sediments exposed in five main regions over an area of 2,000 square kilometres, was not found by accident. In July 1967 Richard Leakey was leading the Kenyan contingent of a multinational expedition that had been organised to explore the fossil potential of the Lower Omo Basin in southern Ethiopia. The area allotted to the Kenyan team turned out not to be as rich as that allocated to the French and US contingent who have since successfully jointly explored the exposed sediments known as the Shungura Formation on the west bank of the river. Leakey, however, realised that in flying from Nairobi to the fossil sites in Ethiopia he had flown over similar looking exposure

some several hundred kilometres further south on the north-east shore of Lake Rudolf. So, following a short aerial reconnaissance in 1967, the first East Rudolf Expedition was mounted by Richard Leakey in 1968. It explored the area as far as it could and found enough vertebrate fossils, including four hominid specimens, to encourage the National Geographic Society (staunch supporters of Drs Louis and Mary Leakey in Olduvai Gorge) to support a more extensive field programme the following year. Their faith was rewarded by further hominid finds, among them a complete cranium, and stone artefacts that appeared to be eroding out of one of the volcanic layers. Dating evidence indicated that the fossils were being collected from sediments laid down between 1 and 4 million years ago, with the younger localities tending to be in the north of the area and the older ones to the south.

Leakey had now moved to the National Museum in Nairobi. The museum authorities recognised the scientific importance of the work and since then the field expeditions have been organised from the museum, which has provided vital technical assistance and considerable logistic support. Despite the fact that many of the scientists involved are from Europe and the USA, the essentially Kenyan identity and basis of the expedition has never been in doubt.

For the 1970 season Leakey sought the assistance of Professor Glynn Isaac from the University of California. With Leakey leading the field team and the two acting jointly as scientific coordinators, they recruited geologists and palaeontologists and, with the continued backing of the National Geographic Society and a generous grant from the National Science Foundation (NSF), they began a planned programme of prospecting, excavations and earth sciences field work. Specialists were recruited to examine the fossils, both hominid and non-hominid, and by this time the scale of work necessitated the establishment of a permanent field camp at East Rudolf at Koobi Fora.

Like the creatures it set out to study, the expedition underwent its own evolution. As the involvement of the participating teams became deeper, and the importance of the laboratory and experimental support that was necessary for the field work was recognised, the East Rudolf Research Project was formed. This consolidated the close relationship of the research with the National Museum, and by means of a research council provided the museum with a panel of scientists which could advise not only on how best the current research could be prosecuted, but which

would also help to formulate future research policy for the area.

Research teams now come from both the USA and Europe to participate alongside the scientific staff of the National Museum. Each team is grant aided to a greater or lesser degree. The NSF in America and the Royal Society, the University of London and the National Environmental Research Council (NERC) in the UK have all provided support for research workers from many disciplines.

The maintenance of research teams many hundreds of difficult miles from the nearest town calls, however, for considerable basic logistic support in addition to that provided for by the research grants. Drinking water is several hours drive away from the main camp and a lorry is used solely to fetch water and firewood. Another lorry plies the barely passable tracks and roads to and from Nairobi with petrol and supplies. The buildings in the permanent camp have to be provided and maintained in an almost constant gale. To supply and maintain contact with small camps spread out over an area of thousands of square kilometres an aeroplane has been purchased which has since proved itself invaluable. To keep the twelve vehicles and two boats that are used in the field in good order a small garage has been built and for the field season a mechanic is resident at the main camp at Koobi Fora.

The responsibility for finding the funds for all these basic facilities has been accepted since the outset by Richard Leakey. Much of this money has been raised in the USA, a good deal of it from proceeds of lectures which serve the dual function of informing people of the research work

and raising the necessary funds. Paradoxically it is this willingness of a scientist to ask the public directly for money, instead of receiving it anonymously from the people through government and research grants, that seems, to earn the displeasure of some fellow scientists.

It is now a fact that the discovery of a possible ancestor of man several million years old is newsworthy. It is apparently also vexing that Richard Leakey, like his father before him, has a knack of exploiting this news to the advantage of his fund raising, and moreover doing it with a style and self assurance that is normally only associated with unravellers of double helices.

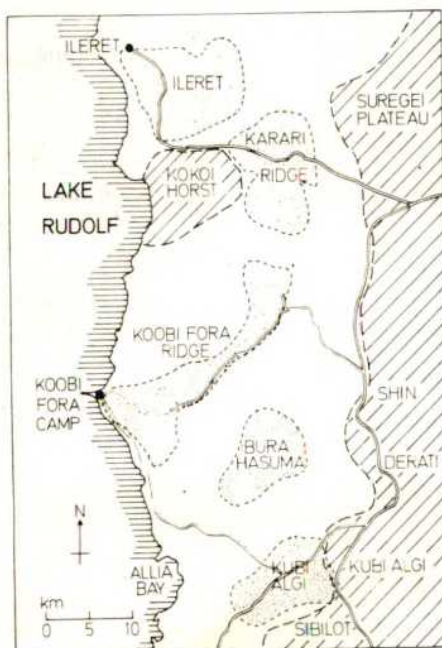
A more pertinent criticism of research programmes into human origins is that the very publicity surrounding the finds tends to concentrate judgement solely on results in terms of specimens recovered. There is an increasing tendency to judge sites according to their position in a hominid league table, with 'oldest' on one axis and 'greatest number' on the other. One would hope that, when scientific judgement is passed on these programmes, it will be on the basis of the quality of the scientific research and on the ability of the research workers to see beyond the immediate problems of the age and nature of the specimens. There is every sign that those bodies that support the research at East Rudolf appreciate the importance of the wider aspects of the research, while at the same time encouraging work on the fossils themselves.

Perhaps it is an encouraging sign of the maturity of hominid research that although research into the geological and palaeoenvironmental context of the material will be maintained at East Rudolf, and in some areas intensified, prospecting for hominid fossils has been temporarily suspended. This to give time for the sample of more than 120 hominids that have been collected to be assessed and analysed.

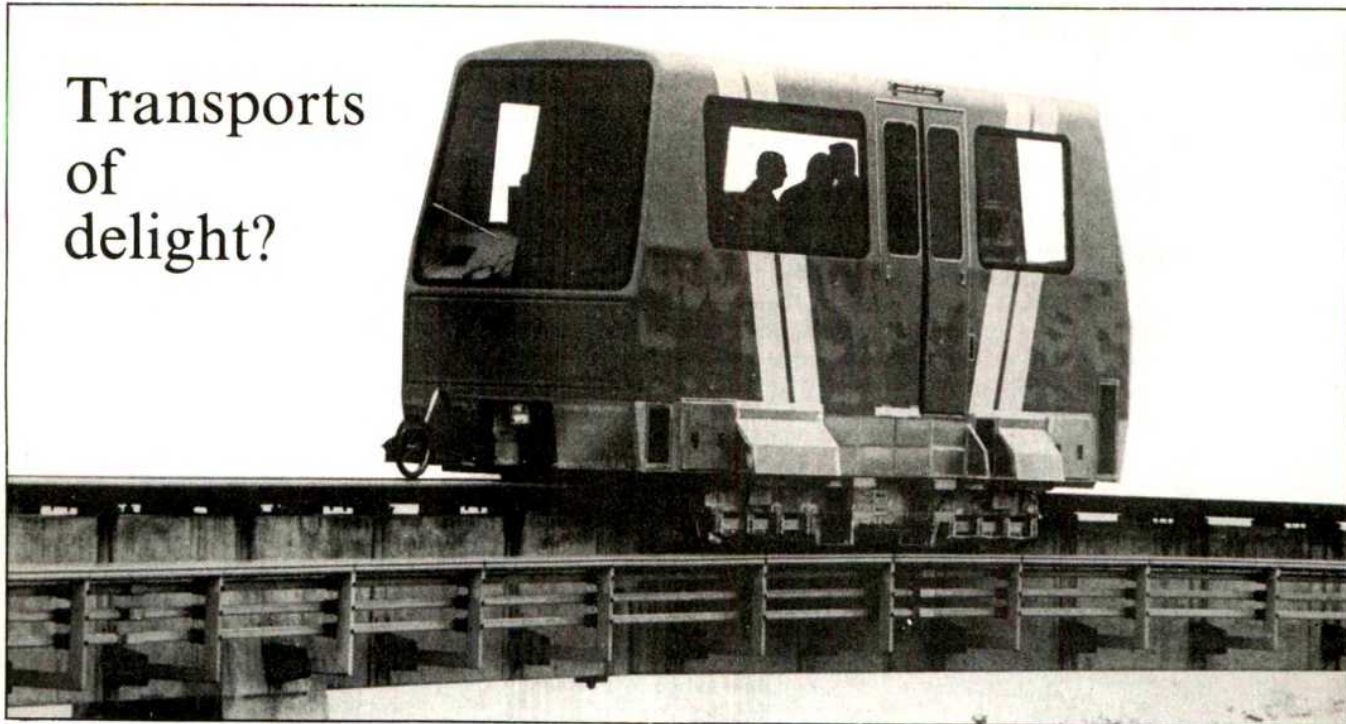
Only when the hominids are considered as just one faunal component, albeit an important one, of an evolving sedimentary basin, as is beginning to be the case at East Rudolf and at other East African sites, will hominid research this decade in Africa have shown itself to be a worthy descendant of Dart's pioneering efforts. That so much progress has been made, both in terms of available fossil material and in the manner in which its context is being studied, is to a large degree because of the scientific and organisational skills of two generations of the Leakey family.

A new centre for prehistory at Nairobi is to be called the Louis Leakey Memorial Institute. □

Fossil site, showing areas of exposed sediment



Transports of delight?



The two-car family on a half-acre plot, in an isolated suburb is no longer considered ideal in Canada, where there is new thinking on the inter-relationship between transport and a better life. Angela Croome reports.

INNOVATIVE transport is at the heart of urban planning. If anyone doubts this it is not Canada's metropolitan authorities. In particular the richest and fastest growing province, Ontario, has put its money (half a billion Canadian dollars since 1971, and an estimated \$1.3 billion over a ten-year period) on this approach to achieving better living. Furthermore, there is confidence that by pioneering a new type of urban transit system and the urban planning philosophy on which it is based, Canadian concerns will soon be supplying most cities of America (North, South and Central) and will acquire for Canada a major new industry and sphere of expertise.

Last year more cars than babies were produced in Canada. At the same time the country is 'urbanising' at a rate of 4% a year, so that if present trends continue, 73% of the population will be concentrated in 12 metropolitan areas by the end of the century. A combination of the need for economies in the use of space and energy and an increasing dissatisfaction with suburban commuter living based on the car has endorsed a radical change in urban planning policy. This was initiated by the Ontario government as far back as 1969 but was underscored in 1971 by a now historic decision to cancel an urban thruway (for motor traffic) in favour of investment in better public

transport. In 1973 the Ontario government succeeded in pegging public city transport fares while improving services by offering local authorities massive subsidies to promote re-equipment and replacement (75% of cost) and reimbursing 50% of the losses on public transport operations. This move has already been reflected in increased public transport 'ridership' figures in most communities.

The problem is seen as "planning better communities with built-in mobility and closer jobs for everyone". "The two-car family on a half-acre plot in an isolated suburb is seen less and less as the good life". Thus says the eloquent and busy F. W. Foley, President of the Urban Transportation Development Corporation based on Toronto. Mr Foley spends much of his time putting across the new thinking on the inter-relationship between transport and a better life in cities. Toronto, the provincial capital, is the focus of these experimental developments. This is appropriate enough, as it has the reputation of being one of the most attractive and best-run of large American cities—and it is at the centre of a region of almost frighteningly rapid growth. It could get itself into the kind of jam that has long since engulfed New York and may be sampled on a smaller scale on the London-Essex margin of the Thames.

Toronto is set to be the demonstration city where the new approach can be tested in practice and where people may come and see for themselves. (The need to keep public reaction in step at every stage is wisely not lost sight of.) Already it has introduced a dial-a-minibus service in outlying districts

Krauss-Maffei TUO-3: withdrawn

where the travelling public is too small to justify a fixed-route service or an underground rail connection. Staggered working hours have already been introduced in city centre offices with excellent results. For some time a city task force has been taking an overview of Toronto's urban planning and transport situation, and involving the public in decision-making. The large Exhibition Park on the city's lakeshore has been picked for the demonstration of the unconventional intermediate transit system labelled "GO-urban" that is at the centre of the planners' thinking. With a track circling the park it will provide links for visitors between the various exhibition halls while at the same time testing elevated track, in tunnel operations, driverless running, computer control, 'ride' comfort and the public reaction to all these elements.

The most significant step, however has been the recent establishment with a Toronto headquarters of the Ontario Urban Transportation Development Corporation (UTDC) with a national and international remit. It is a body without parallel in Canada, and perhaps anywhere else. It is a business institution created by special Act of the Ontario legislature enabling provincial investment to be made in research, design, development and production of public transport systems on a commercial basis. Its role combines that of a think-tank in the transport field with an investment and licensing business somewhat analogous to Britain's National Research and Development Corporation (NRDC). Its immediate object is, simply, to produce "new

transit technology". Provincial Ontario's investment in transport equipment in the next decade alone is forecast officially at over \$1,000 million Canadian dollars; it is already spending nearly \$10 million a year on technology and operational research in this field. The federal government in Ottawa is watching the Ontario initiative and its UTDC with close attention. The province of Alberta has already invested in the corporation.

The key element in the transformation of urban living that the Ontario authorities seek to achieve is the intermediate transit system, using unconventional design. Broadly the object is to obtain the speed and accessibility of an underground (subway) system, but in an overground context with the greater comfort associated with medium density travel—20,000 to 30,000 people in each direction each hour—without the environmental blemishes of noise, fumes and massive land use, and at a fraction of the cost per mile of tunnelling or motorway construction. A world-wide investigation of the state of the art in rapid transit development was completed in 1972 and eight systems were identified for follow-up.

The Krauss-Maffei "Transurban" and Hawker-Siddeley Canada concept (with collaboration from two British companies) were taken up as detailed projects and the Krauss-Maffei system was chosen in Spring 1973. A kilometre-long test track with curves and gradients was completed at the Krauss-Maffei facility at Munich while preliminary site work was started at the Exhibition Park in Toronto. The investment was worth \$30 million but with tough 'break' conditions.

In parallel with these moves by the province of Ontario, Ottawa's Federal Ministry of Industry, Trade and Commerce secured the seven-man linear motor design team from Cambridge, UK, when Tracked Hovercraft Ltd (one of the firms originally associated with Hawker-Siddeley Canada's project) was closed down by the British government. They went to work for the Toronto company SPAR, specialising in advanced technology and space vehicle systems, and the federal government has made a \$2 million industrial development grant to it for work on linear induction motors. SPAR was obviously going to be the major contender for the motor contract for GO-urban propulsion when things got that far. A test track for running a motor palette in various configurations up to 70 miles an hour is now ready at SPAR's Toronto site. This initiative well illustrates the fundamental approach of the Canadian authorities—a rate and massive effort to absorb and vitalise a new technology and a national asset.

A back door into the supposedly lush US market for unconventional rapid transit systems (its urban and automobile problems being similar) opened last autumn when the giant McDonnell Douglas aerospace company of California announced a licence from Krauss-Maffei to market the GO-Urban system in the USA and its territories, and a collaborative agreement with Ontario's UTDC to participate in the technology and development of the Toronto demonstrator.

Very recently the line-up of interests and participants has changed fairly radically with the announcement that Krauss-Maffei was pulling out of the Toronto GO-urban demonstrator project.

Under the cancellation terms Krauss-Maffei paid \$1,800 million immediate cash compensation to Ontario's UTDC, which in addition has the use of the Munich test track and a number of German technicians for two years, together with outright transfer of the accumulated technology, a royalty-free licence for Canada and a non-extensive licence for the rest of the world.

If anything the withdrawal of Krauss-Maffei has put the Canadians in an even stronger position astride the unconventional rapid transit business at least in North America. A question that British experts have been asking for many months is why did the assessors for the Toronto demonstrator plump for a maglev system. The pause may provide just the opportunity to

move to another combination of drive and suspension while reserving rights in maglev technology for other applications. At the speeds envisaged (up to 50 miles an hour) rubber-wheeled carriages propelled by linear induction motors would be equally quiet and non-polluting. They would also make less demands in electricity (for the levitating magnets have a separate supply). It is noteworthy that a combination of advanced linear motor propulsion with rubber wheel guidance and suspension is the type of palette that SPAR, focally placed in Toronto, is currently concentrating on.

The province of Ontario is spending far more on redesigning its public transport than is being spent nationally anywhere else. Its fundamental 'systems approach' must be admired, if not copied. It will provide a blueprint for other industrial countries with growing urban problems. Whether the pre-occupation with new technology to solve the intermediate capacity urban rapid transit problem will be justified remains to be seen; certainly there is no existing vehicle or system that fulfils the specifications set out. There appears little likelihood of lifting the urban scene out of the doldrums and freeing the citizen from the predominance of the car without dedicated central thinking and action, and here Ontario and Toronto in particular have done the world a service and growing cities everywhere would do well to study these developments. □

Eight developers of promising systems representing a range of new technologies were invited by the Ontario Transport Ministry to make technical submissions. The eight systems provide a good indication of world-wide trends in the use and application of new surface transport technology. Only a Japanese submission is lacking—the Japanese have concentrated their effort and investment on high-speed inter-city links.

System Name	Design concept	Automatic command/control	Suspension	Propulsion
Alden "StaRRcar" (USA)	PRT*	Yes	Rubber tyres	Rotary a.c. motors, hydrostatic drive
Ford "ACT"	Line-haul or PRT	Yes	Rubber tyres	Rotary d.c. motors
Transportation Technology inc. (USA)	PRT	Yes	Air cushion	Linear induction motors
Uniflo (USA)	PRT	Yes	Air cushion	Linear air turbine
Bertin "Aerotrain" (France)	Line-haul	Optional	Air cushion	Rotary or linear induction motors
Urba "30/100" (France)	Line-haul	Optional	Negative-pressure air cushion	Linear induction motors
Hawker-Siddeley Canada (Canada)	Line-haul with off-line stations	Optional	Rubber tyres	Linear induction motors
Krauss-Maffei "Transurban" (Germany)	Line-haul or PRT	Yes	Electromagnetic	Linear induction motors

* Personal rapid transit.

international news

AN unusually volatile mixture of politics and technology surfaced in the Commons in the first week of February. Its implications for the Canadian federal government's energy policy were serious enough, but because the case potentially pitted the strongly independence-prone Quebec government against an equally strongly federalist-minded Government of Canada, the outcome will be watched with great interest here.

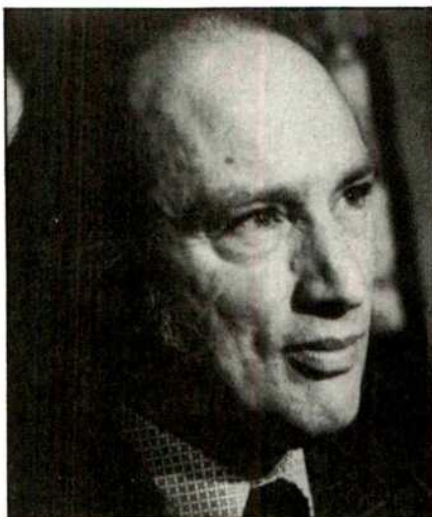
In question was a deal Quebec officials had been quietly negotiating with representatives of France for construction in Quebec of a uranium enrichment plant to be known as CANADIF. This plant would cost from \$3,500 million to \$4,000 million. Because it would use the gaseous diffusion technique, it would require large amounts of electrical power (some 2,500 MW), and this power would be obtained from the James Bay development, an enormous hydroelectric project that itself has been a centre of controversy for some time. (Critics questioned how much of the James Bay power would be used for Canada and how much exported to the United States, and the Indians who lived in the wilderness that had to be flooded at first adamantly opposed the destruction of their hunting ground, then finally agreed after extended legal battles to accept payment of \$150 million and other concessions.)

In order to provide this power, extra capacity would have to be built into the James Bay station at a capital cost of \$1,500 million–\$2,000 million (roughly half the cost of the entire enrichment project). Even then, the enrichment plant would end up using a quarter of the hydroelectricity generated by James Bay—and the cheapest quarter at that. The uranium enrichment project would be used in part to exploit large uranium deposits owned by French companies in western Canada. But the enriched uranium would not be used in Canada, because Canada's own nuclear power system, CANDU, uses natural uranium fuel, unlike the French system. Furthermore, the export of enriched uranium would be used in the reactors of competitors to the CANDU system—perhaps not just the French, but the Americans as well.

All this at a time when Canada has restricted, and proposes eventually to eliminate, the export of energy in the

CANADIF raises energy policy problems

from David Spurgeon, Ottawa



Trudeau: no competition wanted

form of oil from Alberta and Saskatchewan to the United States—and at a time when Canada sees her own capital needs for energy as being approximately \$107,000 million in the next decade and when she has recently become so concerned about the use of her nuclear materials abroad that she has toughened up safeguard provisions at the risk of restricting her own CANDU sales.

The case came to light publicly when a number of documents showing the federal government's concern about the project were leaked to the Press, and Prime Minister Trudeau told the Commons that Canada is not interested in having enriched uranium exported in competition with the CANDU system. He said he had told France and Quebec about this disinclination, but he added that the federal government would still consider the deal if France, for example, wanted to build the plant itself, and if convincing arguments could be presented showing the scheme was in Canada's interests.

Commented *The Globe and Mail* of Toronto in its leader the next day: "It is difficult to see how the uranium enrichment plant proposed for Quebec would significantly enrich anybody except private industry, France and possibly some other foreign buyers."

One aspect that particularly worried critics of the scheme was that the consortium proposing it intended to amortise the cost over a period of only seven to eight years, and to give it a market lifetime of as little as 15 years, despite the fact that its technical lifetime could be 30 to 40 years. This seemed to indicate that they were hedging bets against the much cheaper centrifuge system of enrichment now being pursued by other European interests.

"Is Canada to be used as a colony, to provide supplies for the meantime, and then to be left with an antiquated plant on its hands?", asked *The Globe and Mail*.

The leaked documents showed how worried the federal government was that the Quebec government was trying to put the deal through quickly and quietly, thus presenting Ottawa with a *fait accompli*. This was not the first example of Quebec's attempts to establish a freedom of action usually associated with a sovereign state, and it was not the first example of France's desire to strengthen its influence and ties with francophile Quebec.

One of the leaked documents was a letter from the Federal Energy Minister Donald Macdonald, to Mr Trudeau. He described the consortium proposing the plant as consisting of Canadian Pacific Investments–Cominco (20%), Commissariat à l'Energie Atomique (40%), and the James Bay Development Corporation (40%). He said the consortium seemed to think the federal government's concern was solely with nuclear safeguards.

"The prospect that the federal government might withhold licensing of such a project because of economic considerations had never been contemplated." To show the importance of these considerations, Mr Macdonald attached a memorandum from his deputy minister, T. K. Shoyama, which noted:

- "(1) The federal government would have considerable concern over a large commitment towards an energy source to be utilised for the development of product destined entirely for the export market.
- (2) If there were a shortage of resources in the next decade, the government would want the available ones to be devoted to projects urgently required for the domestic economy.
- (3) For a project to be deemed in the

national interest it would have to be seen to be paying at least the average industrial power rate in the province, not the incremental cost of new power generated by Hydro Quebec, which is expected to be lower.

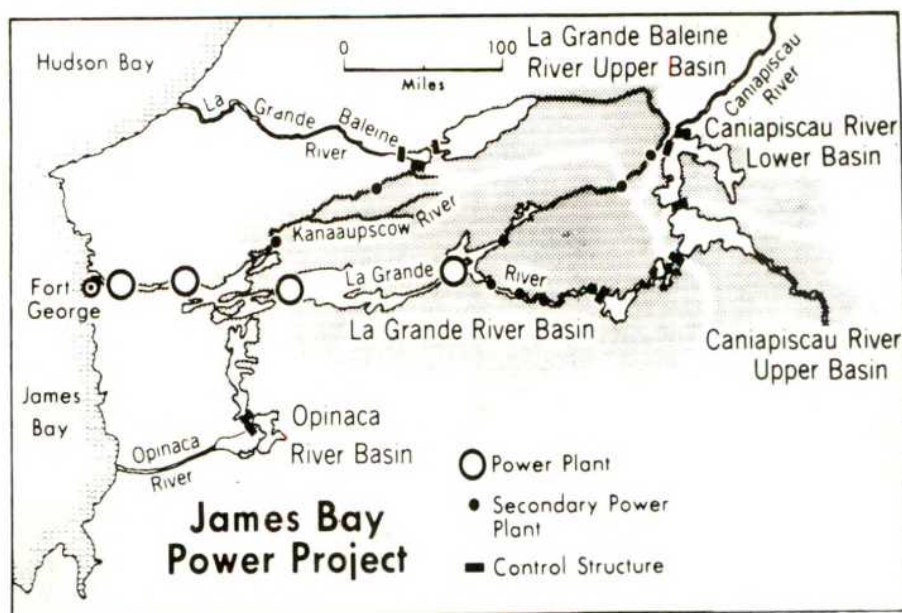
(4) CANADIF proponents wanted contracts for enriched uranium not to be interfered with for the life of the contracts, but 'Canada's safeguards policy could not be subservient to commercial considerations'. A country that might be an acceptable purchaser might later be found to be an unacceptable safeguards risk. If that proved to be the case, then the contract would have to be interfered with."

The memorandum made it clear that the federal officials feared Quebec's Premier Bourassa might try to enter into some commitment with the French government during a meeting he planned in December, and they warned Mr Trudeau and suggested he might speak to Mr Bourassa before that time.

Otherwise "it would be that much more awkward in the future for the federal government to refuse a construction permit through the licensing procedures of the Atomic Energy Control Board".

After his statement in the Commons, Mr Trudeau noted that even if non-Canadian uranium were used for enrichment and export by CANADIF, the federal government would remain concerned because large quantities of Canadian electricity would be required.

The irony of the situation is increased



when one recalls how proud Canadian atomic energy officials have always been in the past of the fact that the CANDU system itself does not need enriched uranium as fuel. For many years they had pointed out that the United States went along the enriched uranium route because she already had a massive investment in diffusion plants for nuclear weapons, while Canada, with no such plants, developed CANDU to exploit her abundant uranium deposits.

Until now, the Americans have been much more successful at selling their reactors abroad than have the Canad-

ians. Now there is the prospect of France exploiting Canadian uranium with the aid of Canadian electrical power in a period of power shortages, and selling it abroad not only to countries that may buy France's reactors but also to the United States.

By the end of the week, Premier Bourassa's only response had been that the CANDU reactor could be modified to use enriched uranium fuel. This is true, but it does not answer the question, why this should be done when CANDU was designed specifically so that it would not need enriched uranium. □

THE company formed to extract oil from the Athabasca tar sands in Alberta, Syncrude Canada Limited, has been saved by an injection of funds from the Canadian, Ontario and Alberta governments. The three announced they would invest \$600 million in the project in exchange for a direct share interest.

Until the announcement, the fate of the project—which aims to tap Canada's greatest potential source of hydrocarbon deposits—was in doubt. Last December, Atlantic Richfield Company of Los Angeles dropped its 30% interest because of mounting costs, currently estimated at \$2,000 million compared with the original estimate of \$195 million in the early 1960s. Shell Canada, which had shown an interest in joining Syncrude, later decided not to.

The remaining participants (Imperial Oil Limited, Gulf Oil Canada Limited, and Cities Service Company of Tulsa, Oklahoma) said they would abandon the project if they could not find additional partners.

So far, about \$160 million has been spent on the scheme. Syncrude scoops up the oil-saturated sand and then

separates the oil by means of hot water and steam. The site is near Fort McMurray, 225 miles north-east of Edmonton.

In return for its investment of \$300 million, the federal government will receive a 15% interest in Syncrude. Alberta gets a 10% interest for \$200 million, and Ontario 5% for \$100 million. Thus, governments will hold a 30% interest and private industry 70%.

Energy critics attacked the federal government's decision in the Commons after the announcement. T. C. Douglas, of the New Democratic Party, said "it represents the greatest sell-out of our natural resources in the history of Canada." He claimed it would benefit no one but the oil companies involved. And there is no way the government can avoid extending similar benefits to other companies, he said. Mr Douglas thought the government, together with other provincial governments that wanted to participate, should take over the project entirely.

Ontario's Premier William Davis sees it differently. He compared his province's investment to the gamble that was taken 100 years ago by those

who invested in the first railways across Canada. But Saskatchewan's Premier, Allan Blakeney, said that the federal government's decision to allow the Syncrude consortium to charge higher oil prices and greater tax deductions for royalties paid to the Alberta government is discriminatory, because his province's oil producers' prices are frozen and they cannot deduct royalties.

Subsequent questioning in the Commons made it apparent that the federal government's investment could climb higher than the \$300 million first promised. The Treasury Board President, Jean Chretien, said Ottawa's commitment is fixed at 15% of the costs, without an upper limit.

But it was not clear how high the investment would climb without question. Any major change in costs, Mr Chretien told reporters, would mean that "all the parties involved will have to sit down and review the situation."

It was also revealed later that Alberta's government will lend Gulf Oil and Canada Cities Service Limited \$100 million each, and will spend \$500 million—\$600 million on a power plant, pipeline and municipal services for the project.

Soviet biologist arrested in Moscow

from Vera Rich

THE arrest of the Moscow biologist Sergei Kovalev on December 27, 1974, seems to mark yet another hardening of Soviet pressure on dissident intellectuals.

Kovalev, whose main scientific work has been in the fields of mathematical biology and genetics (itself, until recently, a suspicious activity, by Soviet standards) is a member of Sakharov's Initiative Group for the Defence of Human Rights in the USSR, and also of a small group of Amnesty International, set up in Moscow in 1974. He has been active in the defence of prisoners of conscience within the USSR, and in campaigning for the free circulation of ideas and information. In 1974, when the *samizdat* "Chronicle of Current Events" resumed publication after an 18 month gap, Kovalev was one of the three dissidents who publicly claimed responsibility for its publication, being convinced "of the necessity for truthful information concerning the infringements of fundamental human rights in the USSR and for its availability to those interested." On the very day of his arrest, in a New Year's message telephoned to London, Kovalev and Sakharov appealed "from a huge and tragic country, the fate of which has huge influence on the life of the whole world" for a general amnesty for prisoners of conscience, mentioning among others mathematician Leonid Plyushch, biologist Vladimir Bukovskii, and psychiatrist Semeon Gluzman. "Let a general political amnesty open for our country and for all countries of the world the way to freedom and a good and sensible life."

The same day, Kovalev was arrested,

apparently on a charge of being in contact with the illegal *Chronicle of the Lithuanian Catholic Church*. This journal, which has a nationalist as well as a religious slant, has already been the cause of arrest of a number of Lithuanians; at the end of November, however, at the request of the Lithuanian KGB, a number of prominent Moscow dissidents, including physicist Andrei Tverdokhlebov, had their apartments searched. Like Kovalev himself, those involved do not appear to be Lithuanians, Kovalev's professional contacts with Lithuanians appear to be slight—only one of his published papers (*Biofizika*, 15, No. 1, 147–155) includes a Lithuanian, I. Dudzyavichyus, among the co-authors. He is, however, reported to have intervened in April 1974, in the case of a Lithuanian woman claiming US citizenship, who was arrested while attempting to reach the American Embassy in Moscow. It would seem difficult, simply on these grounds, to present Kovalev as a fanatic in the cause of Lithuanian separatism.

Nevertheless, the charge is that of possessing and disseminating copies of the *Chronicle of the Lithuanian Catholic Church*. Whether in fact Kovalev did, in his capacity as a campaigner for human rights, have some copies of this *Chronicle* in his possession is not known. The accusation does, however, have a number of advantages for the prosecution. Firstly, the case against the *Chronicle of the Lithuanian Catholic Church* has already been opened, and it is simply a matter of adding another name to the list of the accused, rather than opening a new case. Second, the trial will take place in Vil'nyus, capital of the Lithuanian SSR, away from the focus of attention which a Moscow trial would be for Western journalists. And thirdly, in a society

which extols atheism as "scientific", the attempt to implicate Kovalev with a religious journal may be, to a certain extent, a kind of smearing of his intellectual status.

Nevertheless, these moves to dispel publicity from the arrest and forthcoming trial have not met with unmitigated success. An appeal organised by Academician Sakharov on Kovalev's behalf has been signed by no less than 50 Soviet intellectuals, including linguists, mathematicians and research scientists. □

Toxic chemicals register first step

from Peter Collins, Geneva

BASIC agreement on the establishment and organisation of the proposed International Register of Potentially Toxic Chemicals (IRPTC), was reached at the meeting held at Bilthoven, Netherlands, last month (*Nature*, January 17). Interest in the meeting was considerably more than had been expected by its organisers, the Netherlands government and the United Nations Environment Programme, 57 scientists representing 27 countries and institutions being present. Besides the decision on basic organisation (a central unit correlating and disseminating data from a network of national sources) there was fruitful discussion of the type of work programme that could realistically be undertaken in compiling the register.

For this purpose, there should be an "intensive" programme, providing comprehensive information on a relatively small number of particularly important substances; and a much larger "extensive" programme listing a restricted number of attributes of many more compounds. The intensive programme, it is suggested, should start with a pilot project confined to a small number of chemicals; the object would be to test the best format for presentation of the material, to study means of collecting the data and checking their validity and to estimate the costs of operating this programme. The extensive programme, on the other hand, would be "open-ended", and might start by making use of information from existing data files. Use could be made of the facilities of the UNEP's International Referral System for Sources of Environmental Information (IRS) which is in a position to provide information on the whereabouts and capabilities of existing data collections. This initial survey should cover information of a fairly general nature as well as data on individual chemicals. It should be matched by a survey of potential users of the register, and could thus lead to a preliminary version of the proposed network directory which

"CHEMOSYNTHESIS", the reduction of metals from their ores by means of bacteria, is becoming an important field of research in the Soviet metallurgical industry, and is considered of great potential in the use of a wide range of ores from bauxite to gold agglomerates and uranium ores. Their use of bacteria ranges from the effective utilisation of ores such as the carbonate ores of manganese, where present methods lose up to 35% of the available metal (the chemosynthesis method yields up to 97.5% metal even from low-grade ores), to the extraction of gold and tin from arsenic-bearing agglomerates.

While some such processes are still only in the pilot-plant stage (the extraction of gold, manganese, bismuth, lead, antimony, lithium, or germanium) or

else exist only as theoretical projects (extraction of nickel, thallium, molybdenum, titanium and the microbiological lysis of aluminosilicates), some have already been introduced on a small industrial scale. Such plants, for the extraction of copper, uranium and zinc, are already operating in Kazakhstan and the Urals.

An intensive search is going on, in the research institutes of the Ministries concerned, to find new strains of thermophilic bacteria, multiplying at 55–60°C, which can reduce metal ores. The details of the processes involved remain obscure, but investigations on gold ores have given strong indications that the bacteria and other micro-organisms concerned are capable of forming organic compounds containing the gold in the course of their metabolic processes.

EXPENDITURE by the Department of Industry on research and development has risen 18% to £83 million in the year 1974/5 (*Report on Research and Development 1974*, HMSO £1.10). This barely keeps pace with inflation—even less so when allowance is made for £1 million or so of Rothschild-type transfers to the department in the last year. And when the applications and satellites (expenditure on which, in the form of subventions to the European Space Research Organisation, has risen in a year from £6.9 million to £15.4 million) are removed, the growth is decidedly negative.

Departmental expenditure on exploration and exploitation of the Earth has almost doubled in real terms in the last year to £5.5 million, all the new money going on the marine side. On the other hand civil aeronautics (excluding Concorde), hardly a booming business at present, declines 15% in real terms, getting £17 million of support.

Industry and government establishments now almost equally divide the department's money, industry having increased its share from 42% to 49%. The department's largest laboratories, however, the National Physical Laboratory and the National Engineering Laboratory suffer 10% cuts in real terms.

• Dr Walter Marshall, Chief Scientist at the Department of Energy, was given a rough ride last week by the Select Committee on Science and Technology who were clearly astonished at the lack of progress in formulating a strategy for Britain's research and development into means of energy conservation and developing new energy sources.

The Department of Energy is "still in a thinking period," Dr Marshall told the select committee when asked about the achievements of the multifarious committees that are currently worrying about energy. Dr Marshall and other members of the Advisory Council for Research and Development of Fuel and Power (ACORD) which reviews the research and development of the nationalised energy industries were closely questioned about the specific achievements of this body amongst others.

Dr Marshall said that he expected to produce a strategy in the next few months but that he did not like making promises. The matter of energy research was extremely complex and it had to be recognised that in energy research, the time scales between research and commercial application could be decades.

He was certainly disappointed that the level of spending on energy (other than nuclear power) remained at the present level, and the energy pro-

gramme will demand more money over the years. In the short term, Dr Marshall admitted that conservation schemes would be given priority over schemes to develop new sources of energy as they produced the most immediate savings. He defended the seeming lack of progress since his appointment six months ago by telling the committee that although it was necessary to get going on the energy programme quickly, it must be in the right direction, which meant a great deal of preparatory work.

Round Britain

• Before last year's somewhat abortive International Law of the Sea conference at Caracas, the responsible British minister at the Foreign and Commonwealth Office, David Ennals, held a day-long meeting in London which all interested parties could attend, ride their hobby horses and tilt at their favourite windmills. The Conference reconvenes in Geneva in March with a heightened need for agreements and a convention. On January 30, David Ennals chaired a second get-together at Church House but the level of argument seemed to have been pulled down by the overlong and over-strident proceedings at the international level at Caracas. A disproportionate time was spent on airing the narrow prejudices of the fishing industry presented as facts. The problem of enforcing penalties for oil pollution at sea seemed as far away as ever though a possibly practical suggestion was to invoke the responsibility of the port of origin as

well as the supposed flag of the offending vessel — too often one of convenience.

• The future of the Climatic Research Unit at the University of East Anglia now seems assured. After a period in which the work of the unit was severely hampered by uncertainty about where funds would be coming from over the next quinquennium, the Director, Professor Hubert Lamb, has been able to announce the award of several major grants to his team.

Largest of these is £100,000 from the Wolfson Foundation, covering the years up to June 1979 and "with no strings attached", says Professor Lamb. A Rockefeller Foundation grant of \$120,000 is specifically for a project on mapping and analysing available reports of weather, chiefly from various parts of Europe and Iceland, which cover many centuries in the past. This project will run until October 1977, and it is hoped to extend the analyses back for 1,000 years.

Professor Lamb has stressed the urgent desirability of increasing the number of staff in his unit, and a Nuffield Foundation grant of £24,228 will provide for the appointment of a Deputy Director from March 1976 to December 1979. The unit is also carrying out work under contract for various interested parties, including the City Authorities of Hamburg, who are interested in the incidence of disastrous storm floods in the North Sea, and the Commercial Union Assurance Company, which is supporting a three-year study of changes in the global incidence of tropical cyclones.

The dramatic turnaround in the unit's fortunes is also highlighted by Professor Lamb's future plans. His ambition for the unit to become more interdisciplinary in nature seems likely to be fulfilled, with a Nature Conservancy Council contract for a biologist to study effects attributable to climatic change on the distribution of the natural flora and fauna of the UK and North-west Europe, and "hopes of appointing an economist to keep watch on the impact of current climatic fluctuations on the world food situation, trade and other international aspects".

would be one of the main preoccupations of the central unit.

For positive identification of compounds included in any part of the register, use would be made of the Chemical Abstracts Service (CAS) Registry Number, which is already used by most data collections, although there will certainly be many instances in which this cannot be used.

A major problem may be the coverage of trivial and trade names of substances in languages other than English,

and it is here that international co-operation, especially with manufacturers, will be most necessary.

Consideration was also given to the hardware and software that will be required as the register is developed. One facility that could be of great use is the International Computing Centre, a UN inter-agency facility in Geneva, but it is appreciated that not all storage of data will be computerised, especially in view of the marked interest in the register already being shown in some of the

developing countries.

The presence of scientists from the Soviet Union and Hungary, as well as from Brazil, Ghana, Togo, the Philippines, Tanzania and India, in addition to almost all the highly industrialised countries, shows that the need for this new move in environmental protection is widely appreciated.

With a bit of luck the work of the group will not be hamstrung by the economies being applied elsewhere in the United Nations system. □

A COMMITTEE of the National Academy of Sciences last week shook the foundations of energy planning in the United States by suggesting that domestic supplies of oil and gas may dry up in 25 to 30 years, much sooner than the government has been forecasting. If the committee's estimates turn out to be correct, deep trouble is in store for the much-vaunted drive to make the United States less dependent on imported oil to meet its energy needs.

The estimates form part of a 2-year review of minerals supply and demand, conducted by the academy's Committee on Minerals Resources and the Environment (COMRATE). The committee's central, and inescapable, conclusion is that much more vigorous conservation efforts are needed, not only for oil and gas, but for various other materials as well. As COMRATE's chairman, Dr Brian J. Skinner, professor of geology and geophysics at Yale University, put it last week, conservation should become "almost a religion" if serious dislocations are to be avoided.

Although there is no single "official" estimate of oil and gas resources in the United States, those most commonly used for government planning have been developed by scientists working for the US Geological Survey (USGS). The USGS figures generally suggest that domestic production of oil and gas can be expanded over the next decade, and that supplies will be good for at least 40 or 50 years.

But those forecasts have been challenged recently by much of the oil industry and a number of distinguished experts. They have argued that the USGS's estimates are grossly overblown and that domestic resources are already beginning to dry up. COMRATE's entry into the fray last week with an estimate that broadly supports USGS's critics is likely to sharpen the debate considerably.

The importance of the dispute is this. If the USGS's figures are correct, expansion of oil and gas production in the United States over the next decade or so will be a great help in alleviating dependence on Arab oil. Projections developed last year by the Federal Energy Administration, for example, envisaged domestic oil and gas production going up by more than 50% by 1985, given the economic incentive of high oil prices and some governmental support. But if the USGS's critics are correct, expanded domestic production can be virtually ruled out. Moreover, there will be an acute need to develop alternative fuels (such as synthetic petroleum and gas from coal, and oil from shale) as quickly as possible.

There is virtually no dispute about the size of the so-called "proven" reserves—those which the oil industry

Bad news for US energy planners

by Colin Norman, Washington

has already found and believes it can exploit commercially with conventional technology. The nub of the disagreement concerns the extent of oil and gas deposits which have yet to be discovered—the so-called undiscovered recoverable resources.

In March last year, USGS Director Vincent McKelvey published a set of figures suggesting that undiscovered oil resources in the United States and offshore amount to between 200 and 400 billion barrels, while undiscovered natural gas resources total between 990 and 2,000 trillion cubic feet. By comparison, estimates developed by two oil companies last year put the oil reserves at about 90 billion barrels and gas reserves at about 400 trillion cubic feet. Now COMRATE has come up with a forecast that about 113 billion barrels of oil and 530 trillion cubic feet of gas remain to be discovered.

Surprisingly, those estimates differ little on the extent of oil and gas resources in Alaska and offshore, although COMRATE estimates that fully 70% of future supplies will come from those areas. The chief discrepancy is to be found in the forecasts for the extent of undiscovered deposits onshore in the lower 48 states, and that fact is surprising because the continental United States is about the most extensively drilled area in the world.

The difference is explained by the fact that there are two schools of thought about how the resources should be measured. The oil industry says that the USGS estimates fail to take sufficiently into account the trend of declining oil production per foot of drilling over the past few years, while the USGS says that the oil industry's estimates pay insufficient attention to potential production from small fields.

Until recently, USGS's figures were based essentially on a method which involves extrapolating production rates for oil and gas from known fields to similar geological deposits elsewhere. But that methodology has come under increasing attack, most prominently by M. King Hubbert, a geologist who used to work for Shell, but who now works for the USGS. Hubbert maintained, as long ago as the 1950s, that such predictions are based on production data from the richest parts of existing fields, and thus fail to take into account the fact that the amount of oil produced per well in a given field declines with the number of wells drilled. In other words, the oil com-

panies have creamed off the most productive areas of Texas, Oklahoma and California and information from those areas is unreliable as a guide to what is likely to be found elsewhere.

The USGS's more recent estimates have gone some way towards meeting Hubbert's objections, since they assume that unexplored areas will be only about half as productive as areas which have already been exploited. But Hubbert argues that even those revised estimates will prove to be much too optimistic. A better estimate, he says, is that they will be about one tenth as productive.

Hubbert has at least one important piece of evidence in his favour. In the mid 1950s, he predicted that domestic oil production in the United States would peak in the late 1960s and thereafter would decline; the USGS was then estimating that the peak would not come until the mid-1980s at least.

As it turned out, the peak came in 1970. Hubbert's critics point out that environmental constraints, coupled with a moratorium on offshore oil drilling, may have been responsible for some of the decline since 1970, but Hubbert's arguments have steadily gained acceptance in the oil industry, and in particular they have forcefully been taken up by John Moody, a former official of Mobil Oil and now a consultant to the oil industry. Moody, who was a member of the COMRATE panel, estimated last year that undiscovered oil reserves amount to about 90 billion barrels.

For its part, COMRATE has come close to accepting the validity of Hubbert's approach. Its report states that the projections developed last year by McKelvey "could have been more rigorously derived" and COMRATE's estimate that undiscovered reserve amount to about 113 billion barrels is closer to Hubbert's prediction than to that of the USGS.

Asked what effect he expects the COMRATE report to have, Skinner said last week that "now that the National Research Council (the operating arm of the National Academy of Sciences) has put out a report that comes down in favour of the lower figures, not firmly but at least in favour of them, there will be a lot of pressure on the Geological Survey to justify its methods".

A USGS official noted, however, that oil resources have consistently been underestimated by the oil industry in the past. "Who is to say who is right?" he said, "We are not going to back off from those figures just because they are high". The USGS is, however, updating its estimates and hopes to have a new set of figures, based on the most recently available geophysical data ready by April.

news and views

Io, the anomaly of the Solar System

from a Correspondent

JUPITER is surrounded by an extensive system of 13 satellites. They include four large planetary bodies Io, Europa, Ganymede and Callisto whose sizes are similar to that of our Moon. These satellites reside in the region of trapped radiation around Jupiter and their interaction with the Jovian environment is much greater than the Moon's with the Earth's magnetotail. But it is Io that is the anomaly, not only among the Galilean Satellites but also among all the bodies of the Solar System.

The density of Io, 3.5 g cm^{-3} , is the highest of the Galilean Satellites and comparable to that of the Moon and Mars. In general high-density bodies ($\rho > 3$) are probably rocky silicate structures whereas low density objects ($\rho < 2$) may have a large percentage of ice. During the Pioneer 10/11 flybys, it was found that Io efficiently absorbed electrons in the range 0.16–9 MeV (Fillius and McIlwain, *J. geophys. Res.*, **79**, 3589; 1974; and Simpson *et al.* *J. geophys. Res.*, **79**, 3522; 1974) but had little effect upon electrons of higher energies. Earth-based radio observations had shown that the position of the satellite on its orbit influences the

decametric radio bursts from Jupiter.

Visual astronomers discovered a further puzzling feature. Some reported an increase in brightness of 0.1 mag lasting as long as 15 minutes upon re-emergence of the satellite from Jupiter's shadow though other observers have failed to detect any change in brightness. Does this suggest that the satellite may have an atmosphere? Certainly one would not expect a planetary body with such a low surface gravity to possess a substantial one, so that the discussions of an atmosphere on Io have always been controversial.

But Io does have an atmosphere and an ionosphere too—it is the smallest body in the Solar System with such features. The ionosphere was detected during the Pioneer 10 flyby (Kliore *et al.*, *Science*, **183**, 323; 1974) and was found to extend to some 700 km above the surface with a peak electron density of about $6 \times 10^4 \text{ electrons cm}^{-3}$ at an altitude of between 60 and 140 km on the dayside. A thinner and less dense region was observed on the nightside with a peak density of $9 \times 10^3 \text{ electrons cm}^{-3}$ at an altitude of 50 km.

The diminished nightside ionosphere indicates that this region is produced by the action of solar radiation on the dayside and then decays during the 21 hour Io night.

The Io atmosphere is tenuous, with exotic constituents sodium, calcium, hydrogen, possibly also ammonia and nitrogen, and a surface pressure of between 10^{-8} and 10^{-10} bar. Sodium emission from the satellite's atmosphere provided Brown and Chaffee (*Astrophys. J.*, **187**, L125; 1974) with the first positive evidence for the atmosphere. Trafton, Parkinson and Macy (*Astrophys. J.*, **190**, L85; 1974) reported that the emission came from an extended space around Io. The total column abundance of sodium is estimated to be roughly 10^{11} cm^{-2} in this extended cloud and 10^{13} cm^{-2} in the atmosphere of Io.

As well as the sodium cloud, there is around Jupiter in the orbital plane of Io an extensive cloud of atomic hydrogen (Carlson and Judge, *J. geophys. Res.*, **79**, 3623; 1974). The mean diameter of the torus is about equal to the diameter of the orbit of Io. The torus is not complete, however, but seems to

JOVIAN thunderbolts, occurring at a rate of one per square kilometre of Jupiter's surface every 10 min, may explain the presence of acetylene in the clouds of the giant planet.

Both ammonia and acetylene have been detected in Jupiter's atmosphere, and since both would be rapidly photodecomposed they must be being continuously produced by processes operating in the planet's atmosphere. According to Bar-Nun (*Icarus*, **24**, 86; 1975) ammonia could be produced in the hotter deep layers of the atmosphere and persist for long enough to be carried upwards by convection into the layers where it is detected. But the lifetime of acetylene under Jovian conditions is so short that it must be made closer to the top of the atmosphere.

Bar-Nun's model, which is backed up by laboratory experiments, suggests that the shock waves of thunderstorms are the chief mechanism by which acetylene is produced on



Great Red Spot plastic wrapped by Jovian bolts

by John Gribbin

Jupiter. The electric discharges of lightning will themselves contribute to some extent, but a much greater volume of gas is affected by the thunder,

with the result that significant quantities of methane are converted to acetylene. Ammonia will also be produced in this way, together with hydrogen cyanide, cyanogen and other compounds.

Taking a typical terrestrial storm as a guide, Bar-Nun calculates that $5.3 \times 10^4 \text{ km}^{-2} \text{ yr}^{-1}$ thunderbolts would be needed to produce the amount of acetylene detected. Since Jovian bolts are likely to be more impressive than their terrestrial counterparts, the number may in fact be rather smaller. The compounds produced in these shock waves are almost ideal for explaining the puzzle of Jupiter's colouration, with yellow-brown acetylene polymers and ruby red polymers containing hydrogen cyanide and cyanogen predominating; the correct colour intensity for the Great Red Spot could be produced if thunderstorm activity in the spot is larger than the Jovian average by an order of magnitude.

extend for 60° on either side of the satellite. The lack of emission from that portion of the cloud which happened to lie in Jupiter's shadow suggests that the cloud's diameter is smaller than that of Jupiter and that resonance scattering of sunlight is the primary excitation mechanism.

A torus of this type is thought to be formed by atoms which can escape from the satellite but do not possess sufficient energy to escape from the vicinity of the planet owing to its large gravitational field (McDonough and Brice, *Icarus*, **20**, 136; 1973). Consequently the atoms are bound in closed orbits until lost by ionisation or recapture and tend to produce a toroidal shaped cloud whose density is determined by the ionisation losses. The cloud around Jupiter has a brightness of about 10^4 Rayleighs, composed of roughly 10^{23} hydrogen atoms with a mean lifetime of $\sim 10^5$ seconds. This is slightly longer than Io's orbital period.

But is Io the source of the toroidal hydrogen? The escape of hydrogen from Io poses no problem, since the Jeans escape from an exosphere as cold as 200 K is still very efficient. In order to maintain the cloud the satellite must be supplying hydrogen at a rate of 10^{11} atoms $\text{cm}^{-2} \text{s}^{-1}$. An escape flux of this magnitude would exhaust the entire atmosphere of Io in a few years. Is the surface of Io abundant in hydrogen-rich material?

There are several other puzzling questions regarding the surface of Io, a principal one being of course why it has such strong sodium emissions. Also, why is Io as bright as if it were covered by ice, yet shows no ice absorption features in its spectrum? And why has Io dark poles?

Fanale *et al.* (*Science*, **186**, 922; 1974) suggest that Io's surface composition involves evaporite salt deposits which are rich in sodium and sulphur. (This would both provide a source of sodium and explain the brightness of the planet.) They believe that unlike the other Jovian satellites, Io never had large amounts of ice, yet it apparently was not totally devoid of water like our Moon. As a result of internal degassing, much of Io's water may have seeped to the surface, where it would quickly evaporate as a result of the satellite's proximity to Jupiter and its low surface gravity. The high density of Io and absence of H_2O ice bands in the surface spectrum may mean that this process is essentially completed. Fanale *et al.* speculate that the internal processes are considerably less advanced on Europa and Ganymede, satellites which are apparently very different from Io.

Salt-rich assemblages that Fanale *et al.* propose for the surface of Io are easily derivable from the leaching of carbonaceous chondritic material.

Matson *et al.* (*Astrophys. J.*, **192**, L43; 1974) suggest that the sodium observed in the atmosphere is sputtered from Io's surface by the charged particle bombardment and these atoms could populate the cloud observed beyond the satellite. This mechanism may also be responsible for the calcium emission which Meckler and Eviator (*Astrophys. J.*, **193**, L151; 1974) have observed at 4,427 Å. The previously unexplained colouration of the surface must therefore result from the Jovian magnetospheric fluxes being strongest at the poles of Io.

The presence of ammonia on the surface and in the atmosphere of Io is still controversial, but is required by McElroy *et al.* (*Astrophys. J.*, **187**, L127; 1974) to explain the strong sodium emission. Their hypothesis of the mechanism of sodium emission predicted the ionosphere we have now observed. They suggest that if Io's atmosphere contains $\sim 10^{18}$ molecules cm^{-2} of ammonia, its photolysis would lead to the production and escape of hydrogen at the rate of 2×10^{11} atoms $\text{cm}^{-2} \text{s}^{-1}$. This process represents a net absorption of solar energy and heats the upper atmosphere to around 500 K. Nitrogen would be an important minor product, which when excited by an auroral mechanism could interact with sodium to cause sodium emission. McElroy *et al.* suggest that sodium photoionised by solar radiation may be the dominant source of ionisation. The aurorae possibly resulting in the

sodium emission may draw energy from the current arches to Jupiter, so that significant localised atmospheric heating may result, providing for thermal escape of exospheric sodium into the surrounding cloud.

The Io-controlled radio activity of Jupiter is highly sporadic so that a synoptic patrol of the brightness of the sodium emission may be used to examine the various roles suggested for the high energy particles. But Bergstrahl *et al.* (*Astrophys. J. Lett.*, in the press) found that the resonant scattering of sunlight was primarily responsible for the brightness of the sodium cloud. Auroral processes may still be important for the near surface emission and for heating sodium to its high kinetic temperature. The stability of the cloud over 43 nights of observation implies a remarkably steady source of sodium, since the mean lifetime of steady sodium is less than 16 days.

Io is an anomalous fragment of primordial material bathed in a violent electrical environment which modifies and erodes it. The Jupiter-Io system represents a unique environment where two atmospheres are mutually interacting on a large scale through a magnetic field (for example Goldreich and Lynden-Bell, *Astrophys. J.*, **156**, 56; 1969). Correlated ground-based observations, as well as *in situ* measurements from spacecraft are still required if we are to fully understand this strange planetary body and its interaction with the Jovian environment.

Cosmic rays from the Galaxy

from a Correspondent

THE origin of the high-energy cosmic ray particles ($\geq 10^{17}$ eV) remains a mystery. The long existing controversy on whether the particles observed at the Earth originate inside or outside the Galaxy seems however to be nearing resolution; evidence is accumulating in favour of Galactic sources.

The primary particles are difficult to detect because of their low flux $\sim 1 \text{ km}^{-2} \text{ yr}^{-1}$ at 10^{19} eV. Use is made of the atmosphere as a detecting medium by observing the extensive air showers (EAS) initiated by the primary particles. The secondary particles produced in one of these showers spread out laterally and can be picked up by an array of detectors over an area of several kilometres at sea level. The direction of motion of the original incoming particle can be estimated (typically with an accuracy of about 2°) by timing the sweep of the front of the

EAS across the detector array. By building up statistics over periods of years the distribution in arrival direction of the primaries can be determined.

There are several large EAS detector arrays situated around the world, most notably at Haverah Park, near Harrogate (England), Volcano Ranch (New Mexico), Yakutsk (USSR) and Narrabri near Sydney (Australia). Until very recently the combined results of all the arrays have failed to show up any evidence that was inconsistent with the complete isotropy of the arrival directions of these high energy particles. At lowish energies this is not too surprising as the magnetic fields in the Galaxy can churn up the particles so that on detection their direction of travel bears no resemblance to the direction of their original source.

Sufficient data on the EAS above 10^{17}

eV have now been accumulated, however, and these clearly indicate, for the first time, that there is some degree of anisotropy in arrival directions. By combining together data from the four arrays Krasilnikov *et al.* (*J. Phys.*, **A7**, L176; 1974) have analysed 76 events above 10^{19} eV arriving at the Earth from the northern celestial hemisphere. In an harmonic analysis in right ascension of the arrival directions of these particles a first harmonic amplitude of roughly 40% appears with a maximum occurring about 13 h RA. The authors calculate a chance probability of only $\sim 2.6\%$ for such an effect and add supporting evidence for it being real from data accumulated on lower energy EAS.

Such an anisotropy has obvious importance in connection with the origin of the particles. Hillas and Ouldrige (this issue of *Nature*, page 609) point out that this adds weight to the Galactic origin theories, supporting arguments based on other experimental data including the absence of a cut-off in the energy spectrum below 10^{20} eV due to 2.7 K photons. Plotting the available data on full celestial coordinates Hillas shows that the anisotropy in RA arises from a definite clustering of the arrival directions in at least three regions of the sky. Such separate clustering does not suggest a single distant source but rather a Galactic origin. With this in mind Hillas and Ouldrige further suggest, to explain the total cosmic ray energy spectrum, that the mode of propagation existing below 4×10^{15} eV, consistent with cosmic rays escaping along magnetic field lines, is overtaken at higher energies continuing right up to 10^{20} eV, by a more rapid drift, perhaps radially outwards across the field lines. This suggestion leads to an explanation of the position of the most prominent of the observed clusters. But as the authors point out, to retain nuclei at about 10^{20} eV (the highest energies so far observed) would require a magnetic field extending several kpc from the Galactic plane and even then with a field magnitude of about 2×10^{-6} gauss only relatively heavy nuclei could be contained. There is some other evidence from observations at radio frequencies of the presence of such an extended magnetic field.

Unfortunately it will take several more years of EAS observation to add significantly to the statistics of the anisotropy. Meanwhile at Haverah Park and elsewhere attention is strongly directed towards determining the mass of the primary particles in the energy range $\sim 10^{19}$ eV. Evidence in favour of a high proportion of multi-nucleon primaries at these energies would give good support to the Galactic theories.

Redshifts of BL Lac objects

from R. F. Carswell

BL LAC is the prototype for a class of astronomical objects which has been the subject of a good deal of observational and theoretical work over the past two or three years. Generally, objects of this type are notable for their rapid variations in intensity and polarisation at all wavelengths where these quantities can be observed, and for the absence of any discrete features in their spectra. Some, notably BL Lac itself and, for example, AP Lib and Markarian 501 seem to be surrounded by nebosity and so look rather like galaxies with exceptionally bright nuclei, similar to Seyfert galaxies. Others, such as OJ287, are stellar in appearance and could be related to the quasi-stellar objects, though without any of the emission lines from which a redshift can be determined. In the case of such star-like continuous objects we can only conjecture at their distance from us, since no angular size or redshift can be measured, but for objects with nebosity we can hope to obtain estimates of their distance by looking for observable features in the galaxy component.

Just such an observation has been performed in the case of AP Lib by Disney, Peterson and Rodgers (*Astrophysical Journal Letters*, **194**, L79; 1974) using a number of spectra taken at the Mount Stromlo Observatory in Australia. They noticed that a number of features could be seen very weakly but consistently on their plate material gathered over a period of time, and found that these correspond to lines often seen in normal galaxies if AP Lib has a redshift of $Z=0.0486$. With the currently accepted value of $50 \text{ km s}^{-1} \text{ Mpc}^{-1}$ for the Hubble constant, this corresponds to a distance of about 300 megaparsecs, and the angular size of the galaxy is roughly what we might expect at that distance.

An earlier attempt to find a redshift for a BL Lac object by looking for galaxy features in an extended halo had been described by Oke and Gunn (*Astrophys. J. Lett.*, **189**, L5; 1974). They used a rather different technique, employing a diaphragm to block out the light from the central object so that the galaxy lines would stand out more clearly, and their observations was of the prototype object itself, BL Lac. The absorption features they found were also very weak, but could correspond to a redshift of about 0.07.

There is however considerable doubt about the redshift in this case. More recent observations by Baldwin, Bur-

idge, Robinson and Wampler (*Astrophys. J. Lett.*, **195**, L55; 1975) show no absorption features, and so no evidence that the nebosity around BL Lac is made up of a normal galaxy of stars. They suggest that perhaps one reason for the difference in the results obtained is that a nearby very faint star may have contaminated the spectrum from which Oke and Gunn determined their value. Though the true nature of BL Lac must await further observational work this disagreement between two groups does highlight some of the difficulties involved.

Another BL Lac object, PKS 0735+178, is believed to have an even higher redshift and to be more QSO-like in character. A group of astronomers working in Arizona reported in June last year (Carswell, Strittmatter, Williams, Kinman and Serkowski, *Astrophys. J. Lett.*, **190**, L101; 1974) that a strong pair of absorption lines corresponding to MgII with a redshift of 0.424 had been found in this object. A number of QSOs show sharp absorption lines in their spectrum, always at redshifts lower than, or comparable to, the emission line redshift, and it is believed that in PKS 0735+178 we are seeing the same type of thing. This does not tell us the redshift of the continuum source, however, but it is extremely unlikely that it is much less than 0.424, and probable that it is at about that value.

Apart from placing BL Lac objects clearly well outside our own Galaxy, these redshift determinations can help to test our understanding of the physics



A hundred years ago

THE *Kölnische Zeitung* of Feb. 10 gives an account of Prof. Böhm's (Dorpat) researches on revival after cases of poisoning. He succeeded in reviving cats which had been poisoned by injection of potash salts into their veins, after forty minutes' duration of a state which was in no way different from actual death, the action of the heart and respiration having completely ceased. He obtained these results by artificial respiration and simultaneous compression of the breast in the vicinity of the heart. The professor points out the importance of the latter point, which he deems as essential as the action of the lungs. In any case his researches are of high interest for the relation they bear upon the revival of poisoned persons.

from *Nature*, **11**, 334; February 25, 1875

of such sources. The light and radio emission varies in intensity extremely rapidly; so rapidly that in some cases quite large changes can occur in times of only a day or two. It is very difficult to see how, without signals propagating with speeds greater than light, something can vary on time scales shorter than the light travel time across it, and so this means that the BL Lac objects emit most of their radiation from a region less than a light day or two across. Now if these objects are a very long way away, for example at what might be called 'near quasar' distances, it becomes extremely difficult to understand how the large amounts of radiation required to give the observed brightnesses can be released in so small a volume. Now that we are in a position to say that the redshifts in some cases are quite large, we have the situation where, as Jones (*Astrophys. J. Lett.*, **191**, L15; 1974) and others have pointed out, it seems likely that our understanding of the radiation processes is inadequate, or that perhaps these objects are really closer to us than their redshifts indicate.

Textured superfluids

from P. V. E. McClintock

In a recent communication from the Low Temperature Laboratory of the Helsinki University of Technology, Ahonen, Haikala, Krusius and Lounasmaa describe their transverse nuclear magnetic resonance experiments on superfluid ^3He over a wide range of pressures and temperatures down to 0.7 mK (*Phys. Rev. Lett.*, **33**, 1595; 1974). Their data seem to be consistent with the suggestion by Brinkman, Smith, Osheroff and Blount (*Phys. Rev. Lett.*, **33**, 624; 1974) that the B phase of the superfluid is anisotropic and may be expected to exhibit textures somewhat analogous to those of liquid crystals.

More than 80 papers, mostly theoretical in nature, have been written on superfluid ^3He since the original experiment in 1972 at Cornell University by Osheroff, Gully, Richardson and Lee (*Phys. Rev. Lett.*, **29**, 920; 1972) which led to its discovery. This continuing flurry of activity arises from the entirely novel states of matter which are involved.

It is now known that, below a transition temperature T_c , there are two distinct superfluid phases, referred to as A and B. The phase diagram has been mapped out by various workers and is apparently as shown in Fig. 1. Although, as can be seen, the A phase is stable only within a relatively small area of the P - T diagram, much more is now known about it than about the

lower temperature B phase. The reasons are mainly cryogenic in origin. The two previously existing methods of cooling liquid ^3He —compressional cooling along the solidification line, and adiabatic demagnetisation of the electronic spins of

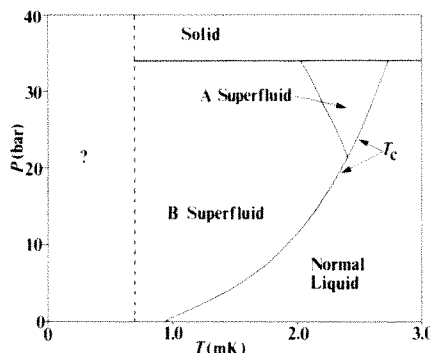


Fig. 1 The phase diagram of liquid ^3He in small magnetic fields at very low temperatures (after Paulson, Kojima, and Wheatley, *Phys. Rev. Lett.*, **32**, 1098; 1974; and Ahonen *et al.*, *Phys. Rev. Lett.*, **33**, 628; 1974). The dashed line indicates the lowest temperature to which the liquid has yet been cooled. Note that the vapour pressure at these temperatures is so small that the liquid-vapour equilibrium line comes within the thickness of the abscissa as drawn here.

cerium magnesium nitrate (CMN)—allow the whole of the A phase to be investigated, but are capable of achieving temperatures only just within the B phase. The technique of nuclear demagnetisation has been applied successfully to cooling liquid ^3He only recently, when the same group at Helsinki reported (*Phys. Rev. Lett.*, **33**, 628; 1974) that they had managed to reach 0.7 mK using this method, thus opening up the temperature range required for a detailed investigation of the B phase of the superfluid.

Why and how does ^3He become superfluid at these low temperatures? It is now almost universally believed, in spite of one or two nagging discrepancies between theory and experiment, that both A and B phases have much in common with the electron gas in a superconducting metal. In each case, the total energy of the particles, whether electrons or ^3He atoms, is reduced if they form so-called Cooper pairs. There is however an important difference in that, for superfluid ^3He , pairs are formed whose relative angular momentum is non-zero, whereas the Cooper pairs of all known superconductors have zero angular momentum. Thus, a Cooper pair in ^3He consists of two atoms orbiting around each other. Much of the experimental and theoretical effort has so far been devoted to investigating how this orbital angular momentum is correlated with the directions of the nuclear spins of the two atoms, and most of the information has been derived from experimental investi-

gations of nuclear magnetic resonance (NMR).

When a single ^3He atom is placed in a magnetic field its nucleus, which has a magnetic dipole moment of its own precesses about the field direction in one of two permitted states, corresponding to two different energy levels. In NMR the resonant absorption of electromagnetic energy at the so-called Larmor precession frequency enables the splitting of the energy levels to be measured. When Cooper pairs are formed, however, the nuclei are no longer acting independently of each other. A careful investigation of the NMR resonant frequency, linewidth and integrated intensity, all of which are found to change from their value in normal, that is non-superfluid, ^3He , therefore yields information about the way in which the nuclear spins and orbital angular momenta are correlated with each other and with possible axes of anisotropy in the liquid.

It seems to have become well accepted that the A phase is in the particular state originally proposed by Anderson and Morel (*Phys. Rev.*, **123**, 1911; 1961) with the nuclear spins of each Cooper pair lined up parallel to each other in the plane of their mutual orbit which has an angular momentum of magnitude $1\hbar$, and with the orbital momenta of all the pairs aligned and perpendicular to an externally applied magnetic field. This implies, of course, that the liquid itself will be anisotropic; that is, many of its properties will depend on the direction relative to the axis of anisotropy in which measurements are made. The Helsinki group's transverse NMR measurements are the first to be made at pressures other than on the solidification line, and it is reassuring to note that their results are in good agreement with predictions based on the assumption of the Anderson-Morel state. In particular, it seems that the pressure dependence of the shift in resonant frequency from its value in normal ^3He is well accounted for by the relation which had been derived theoretically by Leggett (*Phys. Rev. Lett.*, **29**, 1227; 1972) on the basis of that assumption.

It is the lower temperature results of the Helsinki NMR experiments, those in the relatively unknown B phase, therefore, which are in many ways the more interesting. The B phase has been tentatively identified as the state originally proposed by Ballian and Werthamer (*Phys. Rev.*, **131**, 1553; 1963), in which the pair orbital angular momentum is again $1\hbar$, but for which the correlation of orbit and nuclear spins is considerably more complicated than in the Anderson-Morel state. Brinkman *et al.* have been able to demonstrate, however, that it should be possible to define a single axis of

Registry of abnormal karyotypes

AN international registry of abnormal chromosomes has been set up by the Division of Medical Genetics of Johns Hopkins University. Dr D S Borgaonkar and Mr D R Rolling have been gathering data into a computer for the past eleven months and hope to continue to receive details of new and existing human chromosome abnormalities. They aim to establish a sufficiently comprehensive collection to provide information about the prevalence of human conditions, such as Down's syndrome, which are characterised by chromosome abnormality, and to give cytogeneticists a means of exchanging data which might otherwise be lost in their files.

The computer records the name of the person reporting the karyotype, the laboratory where the work was done, the date it was reported, and the arm, region and band of the chromosome on which the rearrangement responsible for the abnormality was found. The registry is being organised on a basis similar to that of Dr Borgaonkar's book *Chromosomal Variation in Man: A Catalog of Chromosomal Variants and Anomalies* (Johns Hopkins University Press, Baltimore, in the press). The book, which was compiled with help from the Institute of Medical Research at

Camden, New Jersey, where there is a repository of abnormal karyotype cultures, and Dr F Ruddle's laboratory at Yale University, consists chiefly of published information. The registry, on the other hand, is being compiled mostly from unpublished data. Print-outs are planned to appear two or three times a year and will be available at cost.

Dr Borgaonkar hopes that, using data in a similar way, it will be possible to catalogue break-points on the mammalian X chromosome of different species to reveal 'hot spots', where unusually large numbers of breaks are reported. He also foresees catalogues of chromosome variation for species such as the common mouse, *Mus musculus*, which has well defined chromosome regions.

The response to requests for data so far has been encouraging. Some regional centres have been set up already, for example in Greece, Belgium, Moscow and Madison, Wisconsin, where reports of new abnormal karyotypes are collated and sent on to Dr Borgaonkar and Mr Rolling.

Their address is Division of Medical Genetics, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.

anisotropy, and that this will tend to lie parallel to an applied magnetic field.

The directions of the axes of anisotropy in both A and B phases are expected to be strongly influenced by the container walls. The reason for this is particularly easy to appreciate in the case of the A phase, for it is intuitively obvious that, unless the orbital plane of a Cooper pair is parallel to the wall, there is a danger of one of the atoms bumping into the wall and so breaking the pair. Pair-breaking requires energy, and so the liquid reduces its total energy if it orients its pairs to rotate about axes perpendicular to the walls. Although the B phase is more complicated, here, too, depairing effects determine that the axis of anisotropy must be perpendicular to the wall. In fact, of course, it is not possible, even by bending the axis, to arrange that it should meet every wall of a given container at right angles unless there exist one or more singularities at which the local anisotropy axis is undefined, and these will presumably position themselves in such a way as to minimise the total energy of the liquid, contributing to a texture which is dependent on the shape of the container.

When a magnetic field acts upon the

liquid in a container of finite size, the texture must be even more complicated. One may, however, assume that the field, provided it is strong enough, will overcome the effect of the walls in determining the direction of the anisotropy axis at positions sufficiently far from the walls: there will be a characteristic distance r_c , dependent on field and temperature, within which the anisotropy direction moves from a wall-dominated to a field-dominated situation. Brinkman *et al.* calculated r_c for the B phase on the assumption of a Ballhau-Werthamer state and found that for $T \ll T_c$ it should be about $10/B_0$ mm with the magnetic field B_0 in mT or, in other words, comparable with the typical dimensions of a pickup coil for low field NMR. This implies that over a significant region of the sample the anisotropy axis will not be exactly parallel to the magnetic field, which will have the consequence of locally raising the frequency at which resonance occurs. The net effect is therefore an asymmetric broadening of the NMR resonance line towards higher frequencies, a broadening which should have a strong negative dependence on the magnitude of the applied magnetic field.

A field-dependent broadening of the

resonance is precisely what the Helsinki group has observed in the B phase, and although they have apparently not been able to check the detailed lineshape with that expected, the magnitude of the broadening seems to be in reasonable quantitative agreement with prediction. It was noted however, that the integrated NMR absorption was significantly lower than the calculated value. NMR measurements were also made on ^3He in the interstices of fine platinum powder, and it was observed that for small fields the signal broadened and disappeared rapidly as T was reduced below the transition temperature. This again is consistent with the picture of Brinkman *et al.* the liquid in each void will have its anisotropy axis determined almost entirely by wall effects because the void dimensions are less than r_c , so that the liquid takes up a domain-like structure with each domain in a different orientation relative to the applied magnetic field. The resonant frequency for each domain is therefore different so that, for the system as a whole, no resonance is seen.

Taking account of the discrepant behaviour of the integrated NMR absorption, and also in view of some earlier measurements of the static magnetic susceptibility, some doubt must inevitably remain as to whether $^3\text{He-B}$ is really in the Ballhau-Werthamer state. Further experiments, in particular measurement of the specific heat for $T \ll T_c$, will be required before the question can finally be settled. There seems little doubt, however, that both of the new liquid phases of ^3He enjoy the curious distinction of being anisotropic, magnetic superfluids which display textures when contained in finite vessels.

Earth wobble, day length and continental drift

from David W Hughes

Two well known facts have recently been combined in a rather fascinating way. The first is the period of Chandler's wobble, the second the secular decrease in the Earth's rotation period. The combination has been carried out by Cannon of York University, Toronto, Canada in a recent edition of *Physics of the Earth and Planetary Interiors* (9, 83, 1974).

The Earth is usually thought of as rotating once a day about an axis which passes through the North and South Poles. The picture is not quite so simple because the Earth is not a perfect globe but an imperfect oblate spheroid with unequal principal moments of inertia. The axis of symmetry of this oblate spheroid possesses

a degree of freedom known as wobble whereby this axis rotates about the space fixed axis of rotation. Now the wobble is very small, the Earth's pole moving around an imperfect circle of diameter about 14 m, equivalent to a 5 s arc axis movement. It can, however, be measured easily by observing carefully the passage of stars across the zenith at an observatory. This gives the colatitude of the observatory to about 0.01 s arc and the pole of the Earth can then be located to 0.3 m.

The Earth responds to the wobble excitation like a damped harmonic oscillator. Spectral analysis of the wobble data shows that part of the variation has a period of exactly one year—driven by the annual forces resulting from the seasonal redistribution of atmospheric mass, water vapour, snow and ice. The remainder of the data has periods ranging from 13 to 15 months, peaking at 428 d—this being known as the Chandler resonance. The length of the year (that is, the frequency of the annual driving force) has remained constant over geological time, whereas the Chandler resonance frequency has monotonically decreased due to the continual loss of terrestrial rotational energy by tidal interaction with the Moon.

Because of the effects of tidal friction on the dynamics of the Earth-Moon system, angular momentum from the rotation of the Earth is transferred to the orbital motion of the Moon. The day is lengthening by about 2.3×10^{-4} seconds each century and consequently the Moon moves further away, the semi-major axis of its orbit increasing by about 4 cm per year. So some time in the past the annual driving force passed through a resonance with the Chandler frequency, resulting in a large-amplitude wobble and during this resonance period geophysically significant amounts of solar energy (the Sun providing the motive power for the annual wobble) were dissipated in the upper mantle.

Resonant conditions would last throughout the time taken for the secular decrease of the Earth's rotation rate to scan the Chandler spectral band. This time interval depends on the rate of decrease of the Earth's angular velocity and on the specific dissipation of the oscillation energy in the Earth's crust. Cannon finds that resonance occurred some time between 242 and 129 million years ago, the mean estimate being 185 Myr ago. It lasted for around 5 to 25 million years. The total energy dissipated during this resonance comes to about 10^{33} erg, of the order of 10^{26} erg yr⁻¹. Considering the variation of specific dissipation with depth Cannon concludes that during this resonance most of the wobble energy was dissipated in the region of

the low velocity zone in the upper mantle, leading to a temperature increase of between 1 K and 10 K. As viscosity is dependent on the exponential of the temperature a 1 K rise causes a 5% decrease in viscosity, a 10 K rise decreasing the viscosity by 50%.

Cannon then goes on to speculate that the Chandler wobble resonance triggered continental drift, the decrease in viscosity enabling the continents to slide on the low velocity zone. Radiometric geological dating has shown that the greater part of the super-continent remained intact for as long as 2,000 Myr before the onset of continental drift. The epoch of the drift onset is thought to be 180–200 Myr ago, agreeing with the time of resonance. Also the Chandler annual resonance is energetically sufficient.

Drift in interference filters

from John Walker

INTERFERENCE filters are used a great deal in optical and spectroscopic instrumentation to isolate narrow regions of the spectrum. That is, they transmit a few selected wavelengths of light, and reflect the rest. One of their drawbacks is that the passband (the region of transmission) tends to shift in wavelength as the filter ages, which can be awkward in some applications. For example, a filter with a passband 0.6 nm wide, centred at 656.3 nm, was required for the H alpha telescope in the Skylab satellite (to study hydrogen in space by means of the H alpha spectral line). Obviously, even a small shift in such a filter would make it useless.

The programme of research which produced the stable filters used in Skylab is reported in a recent publication (Title *et al.*, *Applied Optics*, 13, 2675, 1974). These workers developed a dye laser and monochromator test system which could measure the transmission of the filters and detect the size and position of any defects. Although previous studies had indicated that heat treatment and humidity could cause drift, they found that ten weeks in a 100% humidity atmosphere had no effect on the filters. (One suspects that a longer test period might have caused some changes.) Heat was however found to cause drift.

To stabilise filters the manufacturer bakes them for a few hours at 100 °C. Subsequent storage at temperatures below 38 °C resulted in an average drift of the passband of 0.05 nm per year, but this increased to 2.5 nm above 80 °C, according to Title *et al.* A later set of filters, baked for much longer periods, showed no drift at all at 38 °C, a result in line with earlier work. They

were therefore used in Skylab.

An unexpected observation was that illumination also affected the filters' characteristics, and in a second paper (Title *et al.*, *Applied Optics*, 13, 2680, 1974) Title studied the phenomenon in more detail. A filter which had been exposed to solar radiation showed a drift of the passband towards shorter wavelengths. The transmission profile (as a function of wavelength) also changed, from approximately rectangular to triangular. Tests indicated that solar radiation at about 390 nm was responsible, and when a prefilter was used to cut it out the drift was eliminated. Wavelengths below 340 nm and above 500 nm did not cause any drift. Further experiments showed that longer baking times, which had stopped the 'ageing' drift, made no difference to the illumination effects.

An interference filter consists of alternate thin films of dielectrics of low and high refractive index, in this case cryolite and zinc sulphide. It is in fact a type of Fabry-Perot interferometer. Since zinc sulphide forms the interferometer cavity, and since it absorbs light at wavelengths below 500 nm whereas cryolite does not, the probability was that it rather than the cryolite was being affected by the illumination. This was confirmed by computer calculations, which showed that a decrease in the optical thickness of the zinc sulphide would not only shift the passband in the required direction, but would also result in a triangular profile, as observed.

Title did not determine whether the decrease in optical thickness was caused by a decrease in physical thickness or by a change in absorption coefficient of the zinc sulphide. Earlier work (Schneider and Rauber, *Solid State Commun.*, 5, 779, 1967, and Leutwein, Rauber and Schneider, *Solid State Commun.*, 5, 783, 1967) suggests the latter. These workers studied the optical properties and electron spin resonance of zinc sulphide crystals. Specimens which had been annealed in molten zinc showed a purple colouration, due to absorption bands at 430 and 545 nm. The colouration was enhanced by ultraviolet illumination at 355 nm, with an accompanying reduction in an absorption band at that wavelength. This is evidently the process occurring in the interference filters. Leutwein *et al.* also observed that the effect was reversible. The 355 nm absorption band was restored, and the other two eliminated, by illumination at 436 nm or by heating to 300 °C in the dark. The changes were accompanied by photoconductivity. Obviously there are two point defects in the zinc sulphide which are exchanging electrons through the conduction band. One of them, responsible for the 430 and

545 nm bands, seems to be the sulphur vacancy. The other, which causes the 355 nm absorption, was not identified. The presence of halogen or aluminium impurities also affected the spectra observed.

It therefore seems that illumination-drifted filters might be restored to their original condition by suitable illumination and/or heat treatment. Furthermore, it may be possible, by controlling the impurity content of the zinc sulphide, to make interference filters whose passband does not drift under illumination.

Crack theory developed

from Peter J. Smith

THE dilatancy model for earthquake precursors is one which in general terms attracts wide support. But as O'Connell and Budiansky (*J. Geophys. Res.*, **79**, 5412, 1974) point out, there are disagreements over detail which reveal that understanding is far from complete. For example, Nur (*Bull. Seismol. Soc. Am.*, **62**, 1217, 1972) proposed that the precursory decrease in P wave-S wave velocity ratio (V_P/V_S) is due to the opening of new dry cracks in the focal zone of an earthquake, whereas Whitcomb *et al.* (*Science*, **180**, 632, 1973) noted that vaporising the fluid in saturated cracks would have the same effect. Perhaps it would be wrong to describe this as a disagreement, since it is doubtful whether either party would man the barricades to support their respective viewpoints, suffice it to say that here we have two possible mechanisms for the same phenomenon and that it is necessary to determine which, if either (and if not both), actually obtains.

What does seem to be agreed by most people is that the elastic responses of rocks in an earthquake zone are critically dependent upon the presence of internal cracks and pores and whether they are wet or dry. Yet even here the dilatancy models currently available may be incorrect in detail. As O'Connell and Budiansky again point out, current theoretical analyses of the effect of cracks on the elastic properties of solids apply only to systems in which the cracks are assumed to be so far apart that the effect of each crack on the properties of the uncracked material may be determined independently. This was the basis upon which Walsh (*J. Geophys. Res.*, **74**, 4333, 1969), for example, assessed the influence of both dry and saturated cracks, and his analysis was derived, in turn, from the earlier work of Wu (*Int. J. Solids Struct.*, **2**, 1, 1966) and Eshelby (*Proc. R. Soc.*, **A241**, 376, 1957). Actual crack densities in rocks

have seldom been measured or estimated. So are dilatancy models involving low crack densities really valid?

To find out, O'Connell and Budiansky have carried out a new theoretical analysis which puts no initial restriction on the crack density and thus takes into account interactions between cracks. This freedom turns out to be critical. The model itself consists of a solid containing very thin randomly oriented ellipsoidal cracks which may be wholly dry, wholly saturated or a mixture of both. When the elastic properties predicted from the model are compared with the experimental data obtained by Nur and Simmons (*Earth planet. Sci. Lett.*, **7**, 183, 1969), who measured wave velocities in both dry and saturated rock samples at various pressures up to 3 kbar, it becomes quite clear that consistency can only be achieved by relatively high crack densities. For example, at zero pressure dry Westerly granite has a crack density of 0.25, which corresponds to one crack of diameter 12 units per unit volume (for example, one crack with a diameter of 12 mm mm⁻³). The corresponding figures for other dry rocks measured by Nur and Simmons range from 0.15 to 0.6, while in its wet state the Casco granite has a crack density as high as 0.7 (dry 0.4). Thus, all the rocks are extensively cracked and hence not strictly amenable to any theoretical treatment which presumes dilute crack concentrations.

A similar comparison is possible between the characteristics of the O'Connell-Budiansky model and the premonitory behaviour of the rocks in the region of the San Fernando earthquake of 1971—with equally interesting results. As Whitcomb *et al.* have reported, the seismic velocity ratio V_P/V_S decreased from its normal value

some 3–4 years before the San Fernando event and then gradually increased to its initial value just before the shock occurred. The original discovery of this effect was based on small earthquakes which preceded the main San Fernando shock in what was later seen to be the aftershock area and which were recorded at two stations on the same side of the aftershock zone. Subsequent work has shown that a similar result is obtained for small earthquakes occurring well outside the limits of the aftershock area but measured at two stations which have the aftershock zone between them. In other words, precursory changes in V_P/V_S are observed irrespective of whether the waves used to measure V_P and V_S pass through the main shock's epicentral zone or not.

In terms of the O'Connell-Budiansky model, however, the gross similarity in V_P/V_S behaviour does not entirely extend to the physical mechanism behind the variations. Outside the San Fernando epicentral region (original data recorded at stations on the same side of the region), the crack density before the V_P/V_S decrease lay in the range 0.2–0.3 and the cracks were predominantly saturated. Then during the V_P/V_S decrease in 1967 the crack density decreased marginally and the fluid in the cracks vaporised, which is consistent with the interpretation made originally by Whitcomb *et al.* But during the subsequent increase in V_P/V_S the crack density decreased much more noticeably to 0.15 and the cracks resaturated. This reduction in crack density is inconsistent with increased dilatancy upon resaturation, and implies instead a relaxation of strain which allows some cracks to close completely and the rest to relax sufficiently to eliminate the vapour

Getting closer to prediction

from Peter J. Smith

THE United States Geological Survey claims that "significant progress" was made towards earthquake prediction when scientists at the National Center for Earthquake Research in California managed to anticipate a moderate shock which took place on November 28 last year. This event, which had a magnitude of 5.2, occurred between the San Andreas and Calaveras faults about 16 km north of Hollister, California.

Prediction was based largely on precursory deformation of the Earth's crust and changes in the magnetic field. Crustal tilting was first observed about 4 weeks before the earthquake at two locations near what was to be the epicentre. But a "dramatic anomaly"

in the geomagnetic field in the epicentral region was spotted about 6 weeks ahead. Later analysis of recorded seismic data showed that premonitory variations in seismic wave velocity had also occurred.

According to the survey's director, Dr V. E. McKelvey, this is the first time that such a variety of precursory phenomena has been observed for a single earthquake in the United States. He warned, however, that this success should not be taken to imply that routine prediction for public safety planning is now possible. Significantly, he also pointed out that much still needs to be learned about how successful prediction could be used most effectively in reducing hazards.

phase within them. Inside the epicentral region (later data recorded at stations on opposite sides of the region), the pre-1967 crack density was slightly higher than outside but again the cracks were mostly saturated. And again the V_P/V_S decrease was accompanied by a decrease, albeit rather more marked, in crack density. But thereafter the picture changed. The subsequent increase in V_P/V_S was again related to resaturation but was now accompanied by an increase in crack density.

In summary, then, there are significant differences in the precursory processes inside and outside the immediate epicentral zone. Close to the main shock area the sequence is dilatancy-resaturation-dilatancy, whereas further away the sequence becomes dilatancy-resaturation-relaxation. Extensive cracking is common to both regions, however, and extends over a much wider zone than some workers have previously supposed. And again contrary to some previous views, the observed decrease in V_P/V_S is not due to the formation of new cracks but to a change from saturated to dry cracks. Indeed, changes in the saturation state of the cracks are apparently more important on the whole than changes in crack numbers.

Opening up the Universe

from P. C. W. Davies

MOST cosmologists now accept that the Universe began with a bang. But how will it end? The question has been of long-standing interest to both theologians and scientists, though the former have enjoyed greater success in producing answers. In recent months, however, evidence has been accumulating from diverse astronomical sources which consistently points toward a definite scenario for the future of the Universe. Although there is no question of a unanimous verdict at this stage, a movement of opinion among the pundits is becoming perceptible.

All discussion of this matter takes place within the context of the standard model for the Universe. In this standard model, the Universe moves in compliance with Einstein's general theory of relativity. This motion is visible to us as a general pattern of expansion, and because of the apparent large-scale homogeneity and isotropy, this expansion is assumed to be everywhere uniform.

If Einstein's equations are solved for such a uniform model universe they yield a two-parameter family of motions. All of these solutions predict that the expansion began a few billion years ago, when the Universe was in a

very dense condition. The onset of the expansion, expected to be very hot, is the big bang. The past history of the Universe is therefore, in broad outline, fairly unambiguous according to this theory.

As regards the future motion of the cosmos, all the solutions predict a gradual decrease in the expansion rate. Where they differ is in whether the decrease is strong enough to arrest the expansion and bring about recontraction, with the Universe falling back on itself to end up in a bang much like the one from which it originated. The alternative is for the expansion to continue for ever, with the Universe slowly sinking into thermodynamic equilibrium, after which little of significance will occur.

In principle it is easy to decide between these alternatives. Observations of the rate of recession of distant galaxies, seen as they were in the remote past, should indicate how the expansion rate has slowed since then. Alternatively, measurements of the present energy density in the Universe enables the gravitating effect to be calculated (through the general theory of relativity) as to how vigorously this gravitation is slowing the Universe down.

In practice, both types of observation are difficult to perform and complicated by many contentious side-issues. Now a paper has appeared in the *Astrophysical Journal* (194, 543, 1975) by Gott and Gunn from Caltech and Schramm and Tinsley from the University of Texas in which many of the observations and their theoretical ramifications are examined in detail. Gott *et al.* opt for an ever-expanding (or open) universe. Some of the arguments they use were presented by Gunn at the Seventh Texas Symposium on Relativistic Astrophysics (see the report from John Faulkner, *Nature*, 253, 231, 1975).

The authors parameterise their models in terms of the Hubble parameter H_0 (expansion rate at present epoch) and Ω , the ratio of the observed density of energy to the critical density required to collapse the Universe. For an ever expanding Universe, $\Omega \leq 1$. Their figure 1 shows the constraints obtained in their paper on these parameters.

Although H_0 may be measured directly ($30 < H_0 < 120 \text{ km s}^{-1} \text{ Mpc}^{-1}$) the age of the Universe, t_0 (8 to 18 billion years), is a more severe constraint. A direct estimation of the deceleration is complicated by evolutionary effects in both galaxies and QSOs, which tend to result in an overestimation of the deceleration parameter, $q_0 (= \Omega/2)$. To play safe an upper limit of 2 is placed on this parameter.

In contrast, the density (Ω) measurements tend to be underestimates, first because we only see the luminous matter in the Universe (stars, gas) and second because energy density may reside in the form of very low energy radiation (gravitons, neutrinos). Various methods for estimating Ω are critically described in the paper. Three independent estimates of the relative density of galaxies alone (denoted Ω^*) are used to obtain a value 0.05 ± 0.01 , and various arguments reviewed as to why any intergalactic matter would not be sufficient to give $\Omega > 1$.

In addition, a mention is made of recent theoretical work on the production of deuterium by nucleosynthesis in the hot big bang. The fraction of deuterium produced (D/H or ratio of deuterium to hydrogen) turns out to be very sensitive to the present energy density of the Universe. Using the results of measurements of galactic deuterium abundance, it is concluded that remarkably narrow ranges of Ω and H_0 are permitted in which $0.05 < \Omega < 0.09$ and $49 < H_0 < 65 \text{ km s}^{-1} \text{ Mpc}^{-1}$, predicting an ever-expanding Universe by a wide margin. The possibility of galactic deuterium production and nonstandard big bang physics is briefly reviewed.

The authors conclude that the density of the Universe is low, $\Omega \sim 0.06 \pm 0.02$, and a recontraction is ruled out. The most persuasive part of their argument is the fact that an ever-expanding Universe follows consistently from all the different sources of data, whereas to produce a recontracting model, a number of *ad hoc* assumptions are necessary. It is always possible to invoke exotic processes in the big bang, or to conjecture that most of the mass of the Universe is in the form of undetectable gravitational waves or black holes. But such conjectures are extremely hard to falsify with current technology, and seem somewhat contrived.

If Gott *et al.* are right, then instead of the Universe going out in a blaze of glory by recontraction, collapse and final cremation, it is doomed to everlasting frozen stagnation when the stars go out in a few dozen billion years.

Erratum

In the article "Chilling Statistics on Cyprus" by Peter J. Smith (*Nature*, 253, 500, 1975) magnetic vectors are mentioned in the last sentence of the fourth paragraph. This is incorrect and the sentence should read "In other words, on each side of the intrusion zone the chilled margins will all lie in the same direction (they will all be 'one way') and the degree of 'one way chilling' will be 100%."

articles

Reconnaissance Rb/Sr isochron study in the Bergen Arc System and regional implications

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On the basis of reconnaissance geochronological investigations, and the preliminary results of the reinvestigation of the tectono/metamorphic patterns of the Bergen Arc System it is possible to present a series of results which help to clarify the relationships of the rock units involved in this system. These results fundamentally affect the interpretation of the Bergen Arc System in Caledonian reconstructions of the geology of Western Norway.

Arc system This isochron age fits into the general pattern of the Svecofennian age previously recorded in the Baltic Shield⁶, and conforms with both the pattern of Svecofennian ages in the Nordland district⁷ and recent determinations from the basal/gneiss region in the Kristiansund area (Table 2). It also corresponds to the first Laxfordian metamorphism (LM 1) of the Scottish Lewisian⁹. In their original memoir Kolderup and Kolderup³ drew attention to gneissic rocks in

THE rocks of the Bergen Arc System consist of a series of arcuate belts both of Lower Palaeozoic metasediments and metavolcanics and of gneisses including migmatites and the anorthositic rocks of the Bergen-Jotun kindred¹⁻³ (Fig. 1). The age relationships of the various members of the arc system have been a matter of debate and various models have been proposed to explain them. Kolderup and Kolderup³ concluded that all the arc rocks east of the Øygardens gneiss area were of Caledonian development, a thesis supported by Hernes⁴ as recently as 1967. He further concluded that the Bergen Arcs represent a continuous stratigraphical sequence from Precambrian gneisses through late Precambrian metasediments and migmatites (The Minor Bergen Arc and Ulrikens Gneiss), late Precambrian metavolcanics (the anorthosites and associated rocks) into fossiliferous Lower Palaeozoic rocks (the Major Bergen Arc). Hernes supports this proposal by stating that the arc sequence can be correlated with a corresponding sequence in the Møre area. But Kvale⁵, following Reusch¹ and Werenskiöld², was convinced that the anorthositic and associated gneissic rocks represented an allochthonous thrust unit which overlies and separates the Major and Minor Bergen Arcs with their sequence of Lower Palaeozoic metamorphic rocks. Recent field and geochronological studies support Kvale's proposition, though the pattern of thrust tectonics is more complex than he then envisaged.

This account deals with a limited section of the arc system from the western basement gneisses of Sotra to the rocks of the anorthosite complex.

Øygarden region

The basement of the Sotra (Øygarden) region is represented by a series of granitic, granodioritic and tonalitic gneisses with amphibolites. Field studies show that these rocks have undergone several phases of reworking, under amphibolite facies conditions, that involve at least two stages of anatexis mobilisation. Several localities were sampled in this basement region and quartzo-feldspathic gneisses from the north of Sotra give an Rb/Sr isochron of $1,750 \pm 60$ Myr (Table 1), which represents the oldest age yet obtained from the basement to the Bergen

Fig. 1 Generalised geological map of the Bergen Arc System. Numbers refer to data given in Tables 1 and 4 and Fig. 2.

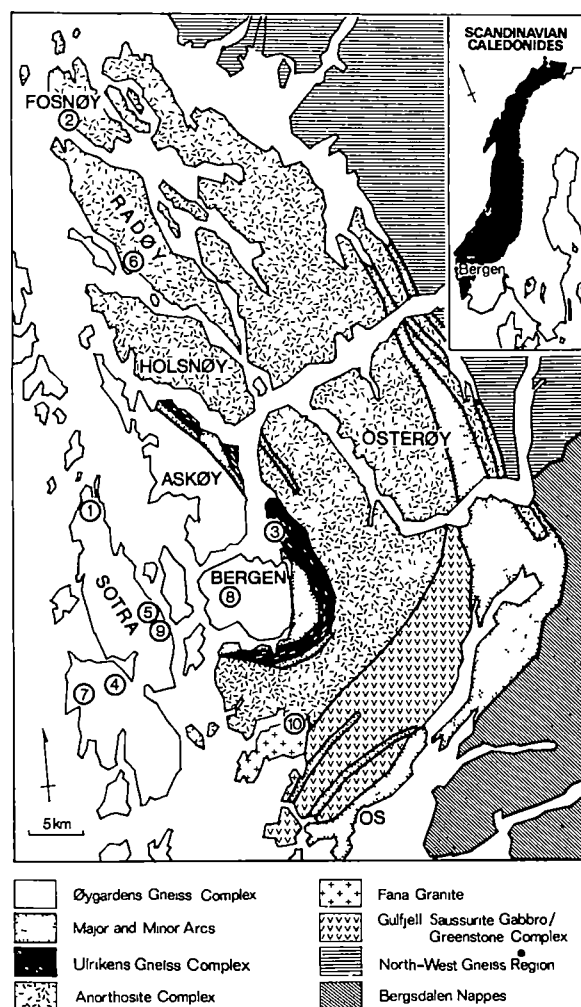


Table 1 Rb/Sr whole rock isochron ages from the Bergen Arc region

Rock type	Locality	Isochron (Myr)	Initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratio (IR)	Source
1 Quartzofeldspathic gneisses	North Sotra	$1,750 \pm 60$	0.7015 ± 0.0020	This study
2 Partially retrograded quartzofeldspathic granulite	Fosnøy	1,775	0.704	Preliminary data
3 Anatectic granite	Sandviken, Bergen	$1,440 \pm 100$	0.7048 ± 0.0026	This study
4 Granite-gneiss	Selsta, Sotra	$1,024 \pm 85$	0.711 ± 0.005	This study
5 Foliated quartz-syenite	Ekrhovda, Sotra	$1,042 \pm 92$	0.718 ± 0.002	This study
6 Mangerite	Manger	$1,064 \pm 24$	0.7030 ± 0.0017	ref 19
7 Fine-grained granite dyke (boudin)	Telåvåg,	800 ± 14	0.731 ± 0.002	This study
8 Intrusive granite veins	Bjørndalstre, Laksevag	890 ± 150	0.730 ± 0.007	This study
9 Medium grained granite	Ekrhovda, Soltra	473 ± 31	0.754 ± 0.002	This study
10 Fana granite	Fana	453 ± 50	0.7063 ± 0.0037	ref 15
11 Rhyolite	Kattnakken, Stord	455 ± 5	0.7071 ± 0.0018	ref 16

the south of Sotra which have the mineralogy of quartzofeldspathic granulites, in the northern Sotra area relict granulite facies assemblages are found in several localities within boudins and show varying degrees of degradation to amphibolite facies. The low initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratio for this 1,750 Myr isochron suggests that this may be an original age rather than a metamorphic age. But on the basis of one isochron all that can be said is that this represents a minimum age for the gneisses, and that the granulite facies relicts could possibly indicate an older event.

In the central part of Sotra and extending onto the mainland at Løvestakken is a later zone of amphibolite facies foliation reactivation. A series of samples were collected from this zone both of the reworked gneisses and of leucocratic dykes and veins cutting them (Table 1). The results of the Rb/Sr isochron study (Tables 1 and 4, Fig. 2) yield the following results: (1) Granitic gneisses from Selsta give an isochron age of $1,024 \pm 85$ Myr and three biotite whole-rock pairs give respectively 598 Myr, 530 Myr, and 402 Myr. (2) A foliated quartz-syenite sheet intruding augen gneisses at Ekrhovda gives a whole-rock isochron at $1,042 \pm 92$ Myr and K-feldspar/whole rock ages of 546 Myr and 550 Myr. (3) A boudined highly radiogenic granite dyke, intrusive into these gneisses at Telåvåg yields a 'two-point' age of 800 ± 14 Myr. (4) Granite veins intruding foliated amphibolites at Bjørndalstre yield a whole-rock isochron age of 890 ± 150 Myr. (5) A boudined dyke of medium grained granite from Ekrhovda yielded a whole-rock isochron of 473 ± 31 Myr, with two biotite whole-rock ages of 398 Myr and 395 Myr.

It is concluded that this zone of structural/metamorphic reworking represents localised effects of Grenville (Sveco-Norwegian) reactivation of the older basement complex. Indeed the two isochron results at 1,024 Myr and 1,042 Myr, with relatively high initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios, conform well with the patterns obtained from Telemark^{13,14} and other west coast regions to the south which lie in the main zone of Grenville regeneration of the Southern Norwegian basement. Similar ages are seen in the nappe areas and the north west gneiss region (Table 3). The 890 and 800 Myr isochrons were determined on later minor granitic intrusions emplaced into this zone after the main Grenville reworking had ceased and again may be compared with similar intrusions from Telemark¹³. They are both now boudinaged, but this is likely to be an effect of Caledonian strains in the area. The youngest isochron age of 473 ± 31 Myr

from a boudinaged dyke is almost certainly an intrusive age and coincides closely with an isochron age of 453 ± 50 Myr obtained by Brueckner¹⁵ from the Fana granite and an isochron age of 455 ± 5 Myr for rhyolitic rocks¹⁶ in the Lower Palaeozoic volcanic complex of the island of Stord, south of Bergen (Table 1, Fig. 2). The deformation of this dyke occurred during the late Caledonian reworking of the basement complex. The Øygarden gneisses were regarded by Kolderup and Kolderup³ as being of Precambrian age, a view restated by others^{4,5}, and indeed indicated from K/Ar determinations¹⁷. The results of the present study abundantly confirm this view.

Ulriken and Manger region

The basement gneisses of the Øygarden area are structurally overlain by the rocks of the Minor Bergen Arc which mainly comprise metasedimentary and meta-igneous rocks of supposed Lower Palaeozoic age^{3,5}, although the Minor Bergen Arc also includes a number of zones of gneissic rocks which have strongly mylonitic fabrics and possibly represent imbricated slices of the underlying basement. As yet no geochronological studies have been carried out on these rocks.

Structurally above the rocks of the Minor Bergen Arc there are the migmatitic gneisses of the Ulriken's gneiss zone, separated from the former by a considerable thickness of repeated blastomylonites. These gneisses have previously been described as Caledonian migmatites³ with associated metasediments. Recent mapping (A. Thon, and T. Sylvester, personal communication) shows that this is a highly complex zone of gneissic rocks comprising at least three thrust units of migmatitic gneisses separated by zones of younger metasediments and blastomylonites. The migmatitic gneisses show a complex history of repeated migmatization separated by deformational phases and periods of emplacement of minor basic intrusives as dykes and sheets. To make a preliminary assessment of the age of these gneisses samples were collected from a series of well-marked anatectic veins which cut foliated basic sheets. The basic sheets cut earlier migmatitic structures which contain deformed basic schlieren and quartzofeldspathic vein material, thus showing that the sampled veins represent a late stage in the tectono-metamorphic development of the gneisses. The result of the first Rb/Sr isochron study from these rocks yields an age of $1,440 \pm 100$ Myr for the late anatectic veins, and shows clearly that the gneisses of the Ulriken's gneiss zone are of Precambrian age. The age of this anatexial-metamorphic event may conform with a recent result of $1,550 \pm 100$ Myr for the overthrust gneisses in the Hardangervidda area^{18,21} and would seem to correspond to the second Laxfordian metamorphism (LM 2) in the Scottish Lewisian⁹. The possibility that the gneisses of the Ulriken's gneiss zone are considerably older than this must not be ruled out, especially as the isochron of 1,440 Myr was obtained on late anatectic veins which postdate an involved sequence of metamorphic development in the gneisses.

The gneisses of the Ulriken's gneiss zone are separated from the structurally overlying rocks of the anorthosite association by a marked development of blastomylonitic rocks and the two zones appear to represent different structural-metamorphic units, as indeed was indicated by earlier workers in the region^{1-3,5}. The results of an Rb/Sr isochron study of mangerites,

Table 2 Svecofennian-Laxfordian Rb/Sr isochron ages from other parts of Western Norway

Rock type	Locality	Isochron age (Myr)	Source
Leucocratic gneisses	Dovrefjell	1,880	ref 15
Quartzofeldspathic gneisses	Kristiansand	$1,708 \pm 60$	ref 8
Quartzofeldspathic gneisses	Egersund	$1,620 \pm 100$	ref 24
		$1,420 \pm 100$	
Quartzofeldspathic gneisses	Hardangervidda	$1,643 \pm 88$	ref 18
Augen gneisses	V. Agder	$1,451 \pm 53$	ref 20
Granitic gneisses	Hardangervidda	$1,550 \pm 100$	ref 21
Various gneisses	Sunnmøre	$1,682 \pm 70$	ref 33

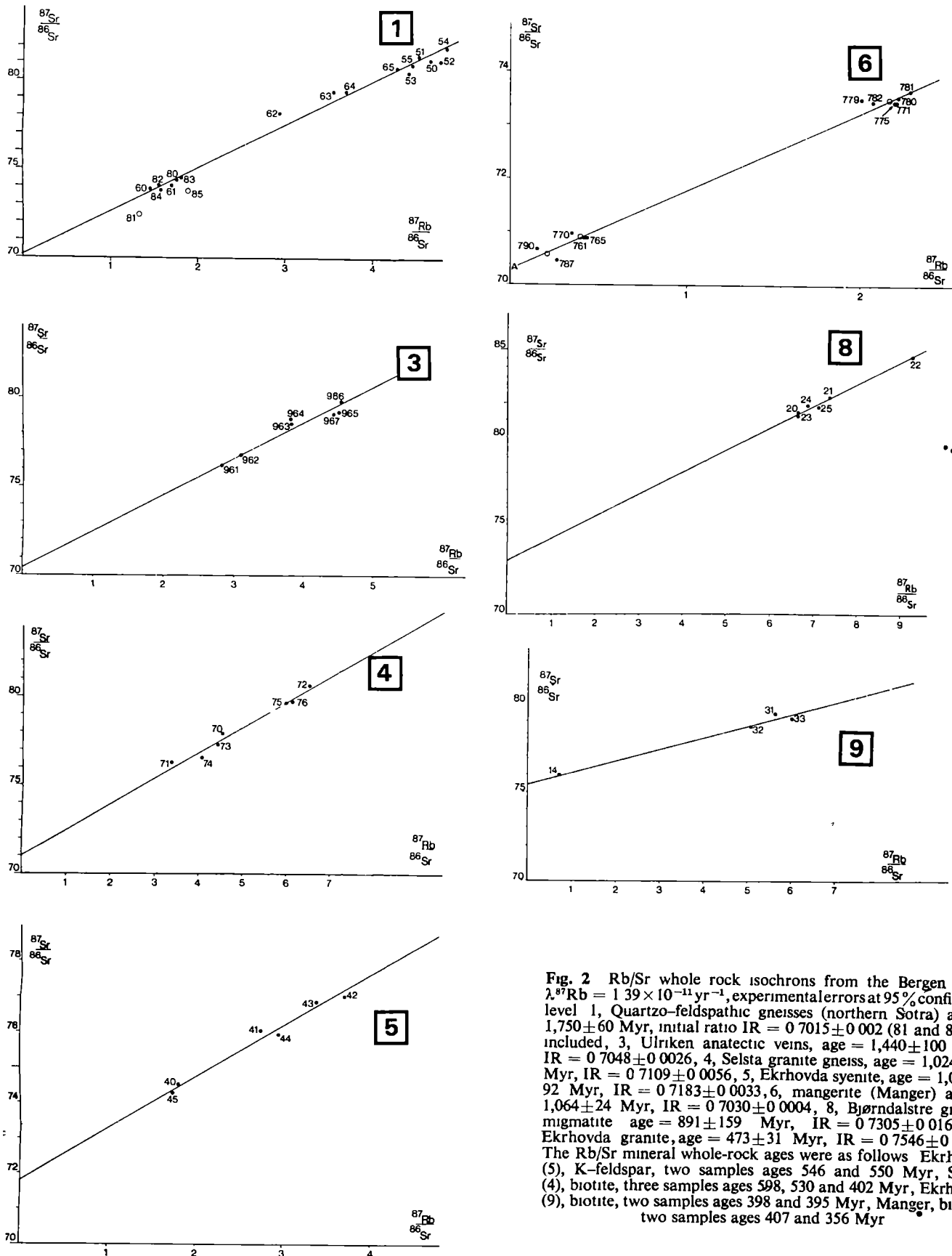


Table 3 Grenville (Sveco-Norwegian) Rb/Sr isochron ages from other parts of West Norway

Rock type	Locality	Isochron age (Myr)	Source
Granite gneisses	Tafjord	1,060±160	ref 35
Foliated granodiorite	Hamlagrovatnet	1,004±100	ref 15
	Bergsdalen		
Granite gneisses	Holsnevatnet	1,253±100	ref 15
	(Sunnfjord)		
Schist and gneiss	Almklovdalen	1,150	ref 15
Granite gneisses	Stavanger	1,160±24	ref 12
Gneisses of Dyrskaard group	Hardangervidda	1,289±89	ref 18
Valldalen supercrustals	Hardangervidda	943±100	ref 18
Hestbrepiggen granite	Jotunheimen	1,033±37	ref 13
Augen gneisses	Kristiansand	1,113±106	ref 11
Late mangerites	Egersund	1,025±13	ref 10
Vrådal granite	Telemark	908±49	ref 13

from the type area at Manger, have already been published¹⁹, and show an isochron age of 1,064±24 Myr which was concluded to represent the age of granulite facies metamorphism of these rocks. This seems to show a Grenville (Sveco-Norwegian) age of metamorphism and fits well determinations made on mangerites in the Egersund region²⁰ both in terms of whole-rock Rb/Sr isochron studies and U/Pb on zircons. The rocks of the anorthosite association have been correlated with those of the Jotun Nappe (as part of the Bergen-Jotun Kindred^{3,5}). No results are available from equivalent rocks in the Jotun Nappe,

but Priem²² has dated the Hestbrepiggen Granite, in this nappe, at 1,033±37 Myr. The work of Banham²³ shows that this granite has undergone deformation and metamorphism. Two K/Ar mineral ages are available on feldspars from pyroxene gneisses at 1,280±30 and 1,020±40 from the north-western part of the Jotun Nappe²⁴. With the limited data available it is only possible to suggest the coeval nature of the metamorphism in the two situations. Two Rb/Sr biotite whole-rock determinations made on the mangerites from Manger and these gives ages of 407 and 356 Myr.

Fosnøy region

Recent mapping of the Fosnøy region in the northern part of Kolderup and Kolderup's Anorthosite association³ shows the geological situation of these rocks is much more complex than has previously been envisaged. Rocks of the anorthosite association *per se* are separated from an amphibolite facies gneiss complex which includes migmatites, metasediments and metabasites, hereafter termed the 'Fosnøy gneiss complex' by clearly marked zones of blastomylonite and porphyroclastic gneiss up to 50 m thick. The structural relationships of the two rock complexes show an intricate folding of the interface including the blastomylonites, by at least two folding phases of presumed Caledonian age, the earliest of which has produced a large sub-isoclinal fold of the contact. The amphibolite facies rocks of the Fosnøy gneiss complex represent strongly downgraded granulite facies rocks. Relicts of the granulite

Table 4 Analytical data for new dates reported. Experimental uncertainties²⁰ are taken as 1.5% and 0.15% for the ⁸⁷Rb/⁸⁶Sr and ⁸⁷Sr/⁸⁶Si ratios respectively (⁸⁷Rb/⁸⁶Sr ratios for all minerals and whole rock samples from Fosnøy, Sandviken and Manger were determined by isotope dilution analysis, the others by XRF analysis)

Sample	Rb (p.p.m.)	Sr (p.p.m.)	⁸⁷ Rb/ ⁸⁶ Sr(At)	⁸⁷ Sr/ ⁸⁶ Sr(At)	Sample	Rb (p.p.m.)	Sr (p.p.m.)	⁸⁷ Rb/ ⁸⁶ Sr(At)	⁸⁷ Sr/ ⁸⁶ Sr(At)
1 North Sotra-quartzo-feldspathic gneisses					5 Ekrhovda-foliated quartz-syenite				
50WR	175.0	106.0	4.64	0.8118	40WR	121.0	188.0	1.80	0.7451
51WR	172.0	107.0	4.50	0.8144	41WR	137.0	141.0	2.73	0.7606
52WR	178.0	105.0	4.76	0.8139	42WR	162.0	123.0	3.69	0.7702
53WR	177.0	113.0	4.39	0.8050	43WR	153.0	127.0	3.38	0.7689
54WR	176.0	103.0	4.82	0.8180	44WR	133.0	126.0	2.94	0.7595
55WR	173.0	110.0	4.43	0.8097	45WR	115.0	184.0	1.74	0.7429
60WR	142.0	276.0	1.44	0.7384	41Kspar	349.0	148.0	6.94	0.7927
61WR	165.0	274.0	1.69	0.7404	42Kspar	352.0	135.0	7.64	0.8005
62WR	165.0	159.0	2.92	0.7813	6 Manger-mangerite				
63WR	166.0	132.0	3.54	0.7945	761WR	92.12	639.7	0.41	0.7086
64WR	172.0	132.0	3.67	0.7945	765WR	92.02	628.3	0.42	0.7089
65WR	181.0	120.0	4.26	0.8077	770WR	76.01	638.1	0.34	0.7094
80WR	109.0	174.0	1.74	0.7435	771WR	188.9	246.2	2.21	0.7350
81WR	97.1	208.0	1.30	0.7244	775WR	188.9	246.2	2.21	0.7350
82WR	97.8	178.0	1.53	0.7406	779WR	180.5	258.1	2.01	0.7354
83WR	112.0	175.0	1.79	0.7455	780WR	185.4	240.7	2.22	0.7360
84WR	122.0	219.0	1.55	0.7375	781WR	206.6	259.4	2.29	0.7370
85WR	132.0	198.0	1.87	0.7385	782WR	183.8	255.1	2.07	0.7335
2 Fosnøy-quartzo-feldspathic gneisses					787WR	173.8	1941.0	0.26	0.7044
7314WR	16.8	243.1	0.20	0.7091	790WR	37.92	788.4	0.14	0.7069
7315WR	37.4	1791.0	0.06	0.7053	761Bi	396.1	43.89	26.2	0.8367
7316WR	16.9	9.571	0.05	0.7056	765Bi	340.8	46.79	21.2	0.8268
7319WR	66.8	1856.0	0.10	0.7065	A Associated Anorthosites				
3 Sandviken-anatectic granite								0.01	0.7032
960WR	172.2	130.6	3.80	0.7869	7 Telåvåg-boudinaged granite dyke				
961WR	165.4	168.7	2.82	0.7612	1WR	358.0	126.0	8.02	0.8250
962WR	160.7	149.7	3.09	0.7670	2WR	288.0	19.5	42.3	1.2084
963WR	174.9	132.2	3.81	0.7846	8 Bjørndalstre-granite migmatite				
965WR	191.8	123.4	4.48	0.7907	20WR	169.0	66.9	6.66	0.8123
966WR	196.0	125.0	4.52	0.7965	21WR	179.0	63.2	7.39	0.8229
967WR	187.8	122.2	4.43	0.7899	22WR	211.0	64.2	9.29	0.8458
4 Selsta-granite gneiss					23WR	164.0	70.1	6.66	0.8141
70WR	196.0	121.0	4.55	0.7793	24WR	159.0	65.9	6.88	0.8187
71WR	207.0	170.0	3.40	0.7623	25WR	172.0	73.1	7.15	0.8173
72WR	225.0	96.0	6.53	0.8065	9 Ekrhovda-granite				
73WR	239.0	152.0	4.41	0.7728	14WR	28.8	118.0	0.71	0.7593
74WR	262.0	181.0	4.09	0.7652	31WR	194.0	101.0	5.62	0.7945
75WR	226.0	106.0	6.00	0.7965	32WR	185.0	107.0	5.07	0.7874
76WR	228.0	105.0	6.14	0.7968	33WR	210.0	102.0	6.00	0.7916
71Bi	861.0	12.4	253.0	2.8467	31Bi	877.0	14.6	192.0	1.8295
72Bi	1134.0	16.6	233.0	2.0741	32Bi	818.0	14.1	191.0	1.8101
75Bi	911.0	12.9	255.0	2.6375					

facies assemblages are variably preserved. The first amphibolite facies foliation in these rocks has been subjected to three phases of folding before the emplacement of a set of granite aplite dykes which themselves predate the formation of the blastomylonites developed at the contact with the rocks of the anorthosite association.

To obtain an assessment of the age of the Fosnøy gneiss complex we collected samples from both the granulites and the aplite dykes mentioned above. Unfortunately both suites of samples have very low Rb/Sr ratios and the dykes in particular have a very small spread of these ratios. On the basis of preliminary results so far obtained from partially downgraded granulites, it is only possible to say that they are suggestive of an age of approximately 1,775 Myr. This approximate age corresponds with ages obtained for the older gneisses on Sotra (Table 1) and indicates them to be of Sveco-Fennian/Laxfordian (LM I) age. Whether or not this represents the age of granulite facies metamorphism or the amphibolite facies retrogression of the granulite facies rocks is not possible to say at this stage of the investigation.

Significance for the Bergen Arc System

The results of the present reconnaissance Rb/Sr isochron study of the western portion of the Bergen Arc System show a series of important results. First, the basement gneisses of the Øygarden are confirmed as being of Precambrian age, with minimum age approximately 1,750 Myr. This corresponds to the main Sveco-Fennian event of the Baltic Shield, though the presence of relicts of down-graded granulite facies assemblages in the rocks of Sotra suggest that this basement complex may be even older. The results further show that the basement of the Øygarden has been reworked during the Grenvillian (Sveco-Norwegian) event at 1,000–1,200 Myr and has subsequently been intruded by granitic dykes at around 800–900 Myr. These results demonstrate the complex sequence of deformational/metamorphic events which have occurred in the basement substrate of the Bergen Arc System before the Caledonian Orogeny. The results from the Ulriken gneiss complex, the Anorthosite association and the Fosnøy gneiss complex show that substantial parts of the thrust nappes overlying the Lower Palaeozoic schists of the Minor Bergen Arc represent overthrust masses of Precambrian basement rocks, and furthermore that they are probably derived from significantly different portions of the basement substrate to the Caledonides. The age of 473 ± 21 Myr for a late granitic dyke in the basement conforms with the age of other granitic activity, during Ordo-Silurian times, within the general region.

The mineral ages are metamorphic ages representing the effect of Caledonian reworking on the Precambrian rocks investigated. The group of mineral ages in the range of 500–590 Myr probably represents either an incompletely reset last event or incompletely reset ages related to the early Caledonian-Grampian event. The group around 400 Myr certainly seems to fit with the well documented late/end Silurian event recorded in most parts of the Caledonides, this is also the stage at which the major transport of the thrust nappes occurred. The latest age at 356 Myr may possibly reflect movements affecting the Devonian rocks to the north of the Bergen Arc region, but inference from one biotite age is only tentative.

Brueckner¹⁵, in reviewing the age patterns from Precambrian rocks in southern and western Norway, makes strong appeal to the lack of continuity of the Karelian-Laxfordian age province in this region, and suggests that there is a lack of fit between the Baltic and Greenland Shields across the continental best-fit reconstruction of Bullard *et al.* This is based on the lack of suitable age determinations in the 1,500–1,800 Myr range south of latitude 62° N in Norway. Since Brueckner's paper, however, several results within this range have been reported (Tables 1 and 2). There are several U/Pb zircon ages within this range^{8,20}, and also K/Ar ages for amphiboles and pyroxenes from the NW gneiss area^{26,27}. Thus it seems that there is considerably more continuity of the Ketilidian-Laxfordian age

province into western and south-western Norway than has previously been suggested¹⁵, though the age pattern has obviously been rendered more complex by the varying intensity of Grenvillian (Sveco-Norwegian) reworking.

On the basis of the Rb/Sr isochron ages at present available it is interesting to speculate on the possible form of the Grenville orogenic configuration in pre-Caledonian times in the North Atlantic region. Traditionally the Grenville-Sveco-Norwegian zone has been projected eastwards from Canada across Scotland into the southern part of Norway, swinging sharply into a NNW-SSE trend in south-eastern Norway and south-western Sweden^{7,28}. Evidence we present here, and other data¹⁵, shows that the effects of the Grenville-Sveco-Norwegian regeneration also affects basement gneisses in the north-west gneiss area of Western Norway (Table 3). The presence of Grenville-Sveco-Norwegian metamorphic ages in the thrust complexes of Western Norway (see also refs 12, 22), which are regarded as having been thrust from a north-westerly direction, poses the intriguing possibility that a major arm of the Grenville orogenic zone previously extended through the northern part of the North Atlantic passing between Greenland and Scandinavia. Several Rb/Sr isochron ages, in the general range 1,000–1,200 Myr have recently been established on the seaboard margins on either side of the northern North Atlantic area. The possibility of an isotopic event at approximately 1,200 Myr in northern Norway has been proposed recently for rocks from the Nasafjell window in Nordland⁷ and from the Lofoten Islands²⁹. In Western Finnmark an age of metamorphism of approximately 1,030 Myr has been indicated for gneisses within the Caledonian belt^{30,31}, and on the western side of the Caledonian orogene Rb/Sr isochrons, K/Ar mineral ages and U/Pb zircon ages in the range 1,060–1,194 Myr have recently been published^{32,33}, for westward thrust basement slices within the Caledonides of the Scoreby Sound region of East Greenland.

Though much more comprehensive geochronological work is obviously required to substantiate this proposition, it seems necessary at least to speculate on the possible presence of a northern arm of the Grenville orogenic zone extending between Greenland and Scandinavia more or less along the site of part of the subsequent Caledonian orogene. The patterns of published age determinations from the continental blocks of Greenland and Scandinavia indicate that the effects of such basement reactivation and metamorphism are restricted to the western seaboard of Scandinavia and within overthrust masses on the respective sides of the Caledonian orogene.

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Missing link between Milankovitch and climate

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A simple empirical model shows that Earth's surface temperature is most affected by seasonal change of irradiation in the interior of North America and Euroasia, and that the highest sensitivity to insolation is reached in the autumn. Past climates are explained by adjustment of the cryosphere to changes of insolation in early autumn.

It has been suggested that the gross changes in Pleistocene climate were controlled by variations in the Earth's orbit, often referred to as Milankovitch's mechanism¹⁻³. Radiometrically dated palaeoclimatic evidence strongly supports such a conclusion. The periodicity in the occurrence of gross cold and warm peaks during the past 150,000 yr matches that of the precession cycle⁴. The process affects only the seasonal and geographical distribution of the irradiation, yearly totals remaining constant. Surplus in one season is compensated by a deficit during the opposite one, surplus in one geographical area is compensated by simultaneous deficit in some other zone. If the mechanism influences climate, a sensitive area and/or season must exist in which heat budgets are modified by changes in incoming radiation to a larger extent than in the rest of the globe and/or during the rest of the year.

Climatologists of the Milankovitch school have seen such a zone in the high latitudes of the northern hemisphere where Pleistocene continental glaciers repeatedly developed and vanished. The sensitive season was considered to be summer because contemporary mountain glaciers would melt during warm dry summers but grow during the cool wet ones. Without further analysis, it was presumed that cool summers would result from lowered insolation and Milankovitch's tables³ as well as their modern counterparts^{5,6} were calculated as time series of zonal irradiation totals for summer half year (spring and summer) and winter half year (autumn and winter).

Today costly mathematical models are used to test the effects of Milankovitch's mechanism on climate^{7,8}. It seems appropriate, therefore, to check the assumption on the decisive climate forming role of summer and examine the geographical relationships of seasonally changing insolation and temperature with recently available weather data. My objective is to localise the area and season which is exceptionally sensitive to insolation change.

Basic assumptions

The ability of the atmosphere and ocean to store and redistribute energy makes an analysis of any local heat budget

rather complicated. We do not have sufficient knowledge and resources to attempt a complete study involving all the elements of the Earth's heat budget on a global scale.

But we can try to identify the sensitive areas and seasons as such, where and when the change in the local ground level irradiation is paralleled with least delay by the largest change in surface temperature.

This approach should be correct if two basic assumptions involved are correct, namely

(1) That the energy transformation at ground level is of primary importance in determining the climatic state, while the variations in direct solar heating of the atmosphere and in the escape of long wave radiation have only contributory effects on the ground level climate⁹.

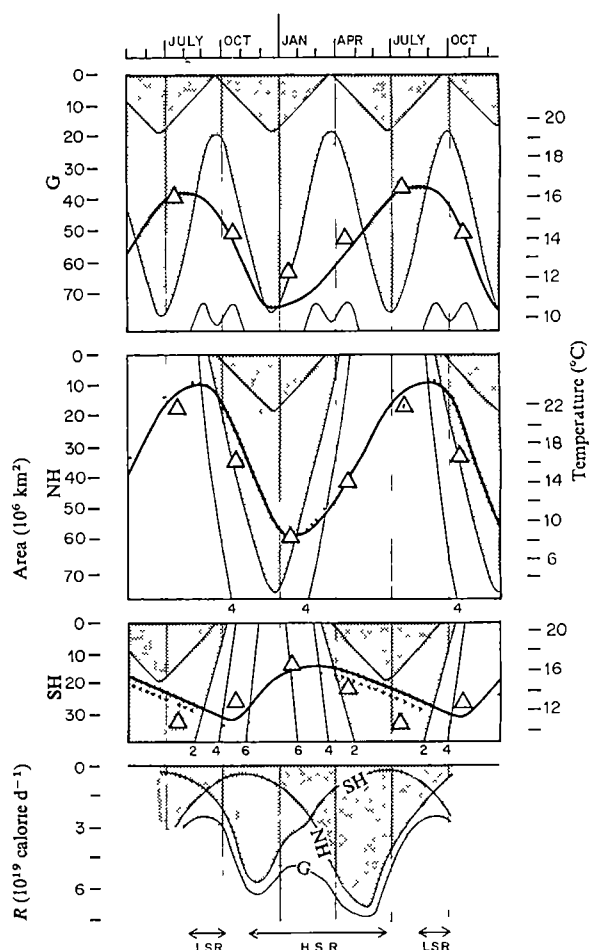


Fig. 1 Mean monthly surface air temperatures (triangles) for the globe (G), Northern Hemisphere (NH) and Southern Hemisphere (SH) compared with extent of cryosphere T_0 (dotted line), snow and ice cover (full line), and Q_0 (heavy dots), Q_{200} (light dots), Q_{400} and Q_{600} areas (4 and 6 respectively). Also shown are seasonal changes in global R (reflection from snow and ice assuming cloudless days). The seasonal wave in mean global temperatures is controlled by the Northern Hemisphere and the mean global and NH temperatures inversely correlate with the extent of cryosphere.

Table 1 Monthly means* of Q , T , and S

	Globe			Northern Hemisphere			Southern Hemisphere		
	Q	T	S	Q	T	S	Q	T	S
	langley	$^{\circ}\text{C}$	10^6 km^2	langley	$^{\circ}\text{C}$	10^6 km^2	langley	$^{\circ}\text{C}$	10^6 km^2
	d^{-1}			d^{-1}			d^{-1}		
January	560	12.2	76	365	8.0	58.4	755	16.5	18
April	570	14.1	59	570	14.2	41.2	570	14.1	18
July	530	16.1	42	725	21.6	14.3	335	10.6	28
October	570	14.3	57	570	16.2	22.8	570	12.4	34
Mean	560	14.2	60	560	15.0	34.8	560	13.4	25

* Q , After Berland¹⁰, T , long term means after Shutz and Gates¹¹, satellite observed mean S areas covered for at least five consecutive days in the Northern Hemisphere during 1967-73 interval and in the Southern Hemisphere in 1968 (ref 14).

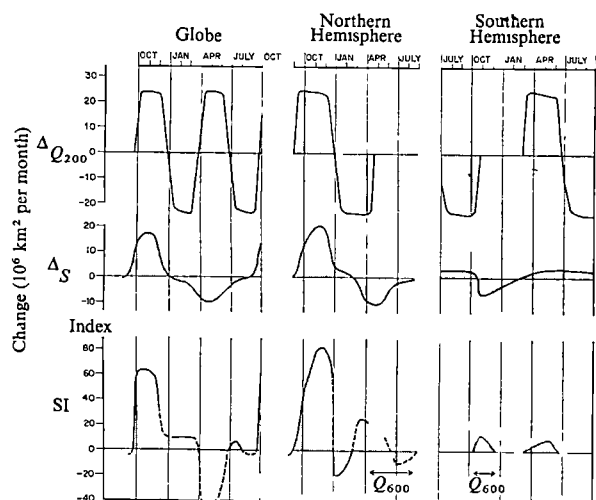


Fig 2 Rates of increase (positive values) and decrease (negative values) of S and Q_{200} areas through the year. Sensitivity index SI shows the proportion between ΔS and ΔQ_{200} after the formula $SI = (\Delta S / \Delta Q_{200}) \times 100$. High positive index indicates high sensitivity of climate to insolation change, as observed in the Northern Hemisphere from September through November. Q_{600} marks intervals where ΔQ_{200} was substituted in the formula by ΔQ_{600} . Dotted line through areas where ΔS or ΔQ approaches zero.

(2) That the atmospheric and oceanic circulation in general tends to smooth and compensate, rather than strengthen the geographic and temporal differences in the heating of the globe. Thus, the longer the delay between the local irradiation change and climatic response, the greater the influence of compensatory processes related to lateral heat transport, and the lower the sensitivity of local heat budget to the insolation input at any moment.

The QTS-1 model

QTS-1 is a highly simplified one-level global climate model in which heat transfer in the atmosphere and oceans as well as the hydrologic cycle are completely disregarded. It is named after the main recognised variables: ground level irradiation Q , surface air temperature T , and the area of snow and ice cover S . The 'instant' climate state in the model is defined by the mean surface air temperature T . Extensive parametrisation of empirical input data was used in the present first version of the model, QTS-1. Improved variants will be studied in the future.

The sources of input data and the way they were parametrised are as follows:

Q Daily totals of solar radiation incident upon the Earth's surface under cloudless sky, accounting for zonally averaged mean turbidity¹⁰. Units are langley d^{-1} , where 1 langley = 1 calorie cm^{-2} . Related measures are Q_0 , the area in million km^2 with no irradiation income, and Q_{200} , Q_{400} , and Q_{600} , the areas where irradiation is below 200, 400, and 600 langley d^{-1} respectively. Cloud effect on Q is omitted.

T Surface air temperature ($^{\circ}C$) (refs 11–13). T_0 is the area with temperatures below $0^{\circ}C$, corresponding roughly to the ground level extent of the cryosphere.

S Snow and ice covered area irrespective of snow density in million km^2 . Data from the northern hemisphere obtained from NOAA weekly maps of average snow and ice boundaries, all other data as listed in ref. 14. Only areas covered for more than five consecutive days are included. Though the reflectivity of snow fields as well as bare surfaces varies within a wide range, the establishment and disappearance of snow introduces by far the most striking seasonal change into surface albedo¹⁵.

R The parametrised measure of radiation (langley d^{-1}) reflected from snow and ice in excess of that which would be

reflected in the same situation by bare ground or ocean free of ice cover.

The principal advantage of the QTS-1 model with immobilised atmosphere and non-existent heat storage capacity is that local air surface temperatures are a function of instantaneous local irradiation and albedo only. Thus, solution of our problem, which is to locate the sensitive zone and season, can be found by determining where and when the observed real world behaviour most closely approaches the relationship anticipated in the model.

Interpretation of QTS variations

The observed data are in Table 1. The globe is warmest in July under low Q and coldest in January under high Q . The global mean temperature is not a direct function of global mean Q , and the seasonal Q wave is approximately symmetrical between the two hemispheres whereas the T wave is not.

Figure 1 shows the variation of global and hemispheric mean T compared to areal changes of T_0 , S , Q_0 , Q_{200} , and Q_{400} . The T_0 area closely approaches the S area, so that for the purpose of the present discussion, T_0 can be considered equal to S . There is an inverse relationship between the global T and S ; in a first order approximation, the global mean T and thus the global mean climatic state could be considered a function of S . The same relationship is valid for the Northern Hemisphere.

The seasonal wave of global T and S is shaped in agreement with the T and S waves in the Northern Hemisphere because of high seasonal contrast over the northern continents. Thus, the seasonal variation of temporal global climate states seems to be basically controlled by variations in snow and ice cover area in the Northern Hemisphere.

The fast global increase of S between September and November coincides with the buildup of snow and with the increase of Q_{200} area in the Northern Hemisphere, and with the sharp increase of R in the Southern Hemisphere, when the planet passes from low surface reflection (LSR) to high surface reflection (HSR) mode. Although the increase of S in the Northern Hemisphere is relatively fast and the decrease slow, the opposite seems to hold true for the Southern Hemisphere where the buildup of fields of pack ice is slowed down by the heat reserves in the ocean. The snow cover, once established with its high albedo and high specific heat required for melting has a self-perpetuating cooling effect on environment.

In Fig. 2 the rates of change in S and Q_{200} areas are plotted

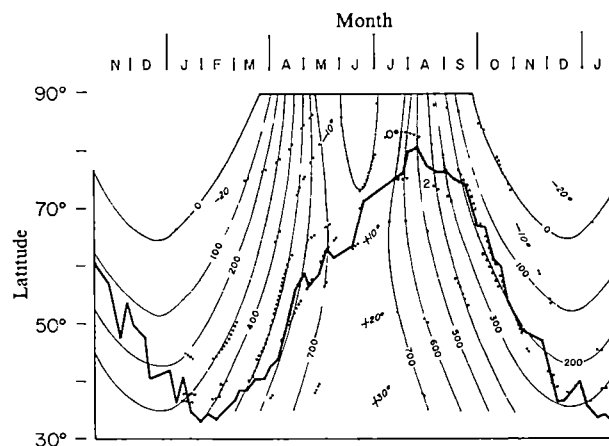


Fig 3 Seasonally changing mean latitudinal position of the snowline (full black line) from October 1971, until January 1973, between $50^{\circ}E$ and $70^{\circ}E$ compared with long term mean surface air temperatures ($^{\circ}C$) (dotted lines, zone between $0^{\circ}C$ and $+2^{\circ}C$), and ground level cloud free insolation Q (langley d^{-1}) (stippled). From September through November the snowline and the $0^{\circ}C$ isotherm follow the 200 langley d^{-1} isotherm. First day of each month is marked.

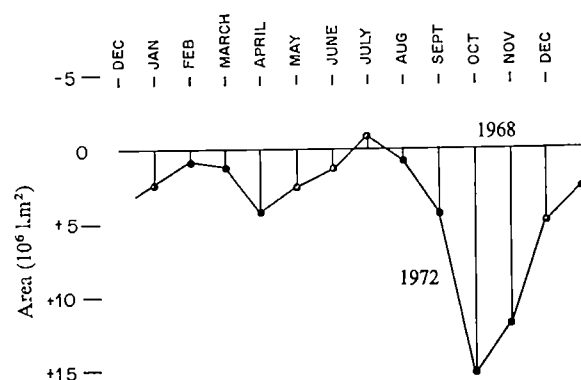


Fig 4 Departure of the monthly mean area covered by snow and ice in the Northern Hemisphere in 1972 (cold anomaly) from 1968 levels

as a function of season. The ΔQ_{200} values are almost the same in both hemispheres whereas the ΔS is greatest in October and November in the Northern Hemisphere. The disintegration of snow fields in the Northern Hemisphere and pack ice in the Southern Hemisphere are substantially slower.

The bottom line in Fig 2 shows the sensitivity index (SI) which indicates the relationship between simultaneous ΔQ_{200} and ΔS . Index values are irrational and are not plotted (denoted by connecting dotted line) if ΔQ_{200} or ΔS approach zero. Large SI indicates that the rate of change of Q_{200} closely parallels that of the S areas and thus the high sensitivity of S to insolation change. The greatest SI for the globe (about 60), Northern Hemisphere (about 80) and the Southern Hemisphere (about 13) are reached simultaneously in October and November, identifying this interval as the sensitive season.

Changing mean latitudinal position of the snowline in the interior of the Euroasian continent and the corresponding T and Q values are shown in Fig 3. They were plotted for the meridional segment between 50° E and 70° E, which is composed mainly of lowland. The advancing snowline in autumn, together with the T_0 boundary, closely follow the isoline with irradiation $Q = 200$ langley d^{-1} .

In contrast, between March and June, during the poleward retreat of T_0 and S , the snowline move is substantially delayed relative to Q and the irradiation above the snowline steadily increases from 400 to about 800 langley d^{-1} . Thus, in the studied segment, a distinct seasonal asymmetry exists in the relationship of Q to S . The snowline essentially follows the irradiation drop in autumn but it is substantially delayed behind insolation rise in spring¹⁷.

Between September 1 and December 21 in the North American and Euroasian interiors, the Q at the snowline drops only by about 2 langley d^{-1} for each degree of latitude of snow fields southward advance. Q increases by about 15 langley d^{-1} (from 10–22) per degree of latitude of snowline retreat between March 1 and June 21. Similar measurements in the Southern Hemisphere give the Q departure at the advancing pack ice front as about 45 langley d^{-1} degree⁻¹. The greatest sensitivity of S to Q (and thus of climate to Q) is reached at the time when the snowline accurately follows the shifting insolation pattern resulting in zero change in Q over the snowline. The conditions observed during autumn in the interior of the continents of the northern hemisphere are closest to this situation.

Test against present weather variability

In the real world the surface temperature and reflectivity depend on a number of factors, many of them unrelated to local insolation. Thus, simple relationships anticipated in the model may not exist at all. On the contrary, feedbacks may exist strengthening the dependence.

• There are several ways the validity of tentative conclusions derived from the QTS-1 model could be tested. One is mathematical modelling which could be undertaken in the future. Others are various partial tests, using the present observed

short term fluctuations in meteorological variables. Such tests could include correlation of past snow cover history with annual temperatures. The present hypothesis would be supported if significantly high correlation was found between the years with relatively extensive snow cover in fall and the anomalously cold years. Satellite observations of snow as yet cover too little time to provide statistically significant tests. Available maps, however, include four relatively normal years (1967–70) and two anomalous cold years, 1971 and 1972. The atmosphere below the 500 mbar pressure level north of 15° N was 0.35° C cooler in 1972 than in 1968 (ref 18). So, at least for the northern hemisphere, the snow and ice cover extent of a 'normal' year could be compared with that corresponding to the anomalously cold year. Figure 4 demonstrates that the 1972 anomaly, as compared with 1968, was indeed characterised by significantly larger area of snow and ice on an annual average and by significantly earlier deposition of snow in autumn.

Test against palaeoclimatic history

If the continents in the northern high and mid-latitudes are the sensitive area, and the autumn is the sensitive season intercepting the extraterrestrial impulses, then the timing of the expected impact of Milankovitch's mechanism on this area and season should fit the radiometrically dated palaeoclimatic evidence.

Figure 5 shows the seasonal variation over the past 30,000 yr in energy flux at the top of the atmosphere in the plane facing the Sun, as a result of the Milankovitch mechanism. The September and October mean global irradiation on the top of the atmosphere peaked about 6,000 yr ago, reaching 725 langley d^{-1} and dropped to a minimum of 675 langley d^{-1} about 17,000 yr ago, with corresponding change of Q values.

It is well known from isotopically dated palaeoclimatic evidence that the highest postglacial surface temperatures were reached about 5,000–7,000 yr ago and that the most recent glacial culminated sometime between 15,000 and 19,000 yr ago¹⁹. In a first approximation the correlation can be extended to at least 150,000 yr BP simply by using conventional Milankovitch summer or winter half-year curves with timing shifted by 5,000 yr (ref 20).

Within the precision limits of radiometric dating the gross shape of palaeoclimate curves for the past 20,000 yr also correlates well with the irradiation levels in early autumn^{4,19,20}. This observation, even though not yet analysed quantitatively,

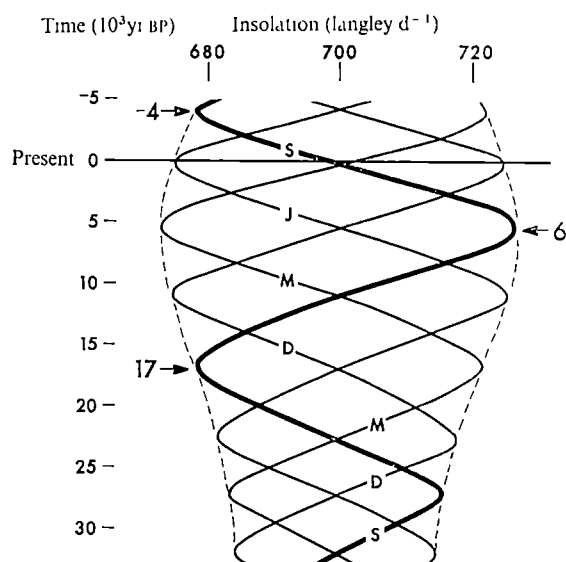


Fig 5 Irradiation at the top of the atmosphere in the plane perpendicular to solar rays during the past 30,000 yr, at the summer solstice (J), winter solstice (D), spring equinox (M) and autumn equinox (S). According to my QTS-1 model, the autumn irradiation, approximately represented by the S curve, controls the global extent of cryosphere and through it the climate.

lends substantial support to my hypothesis, which predicts delayed snow accumulation in the Northern Hemisphere under the high autumn irradiation 6, 28, 42, 55, 77, 101 and 122 thousand years ago with, by contrast, an early snow accumulation in the north and a late pack ice melting in the south under the low early fall irradiation 17, 36, 51, 67, 89, 111 and 134 thousand years ago. It could be speculated that the mechanism was less efficient during full glacial conditions in North America because of substantially reduced seasonal snow fields at the expense of permanent ice. The opposite holds true for Asia and Eastern Europe. So the North American subcontinent could have been less sensitive and Asia more sensitive to insolation change during a full glacial than they are today. Also the timing of the most sensitive interval may have shifted between August and November according to the varying surface properties of the Pleistocene continents.

Because the QTS-1 model does not recognise heat and water transfer in the atmosphere, the validity of the conclusions in the real world remains tentative. But my tentative results indicate that (1) the conventional assumption on summer half-year insolation in the northern hemisphere as linking Milankovitch's mechanism with climate through millenia long delay is probably invalid, (2) an accurate astronomical dating of gross Pleistocene climatic history is feasible since the climate probably adjusts to changing insolation patterns with negligible delay, (3) the long term outlook for natural climate development as predicted from the QTS-1 model is the perpetuation of oscillatory cooling for another 4,000 yr and (4) the heat balance developments in the inner parts of the

northern continents deserve the close attention of long-range weather forecasters. The sensitivity concept provides a plausible explanation of the 20,000-yr climate cycle and a new look at the mechanism of climatic variability. Nevertheless it is far from a complete theory of climate change and leaves unexplained the causes of the 10^6 yr Pleistocene glacial cycle as well as the causes of climatic variability on the time scales of millenia.

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Darwinian evolution in the genealogy of haemoglobin

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Frequencies of mutations between reconstructed ancestor and descendant sequences of codons for metazoan globin chains show that natural selection guided protein evolution. Mutations which improved haemoglobin function were accepted at an accelerated rate in the first vertebrates. Rates decelerated after functional opportunities had been exploited.

THE differences between 55 contemporary globins with well analysed amino acid sequences¹⁻⁴⁷ have been used to deduce the genealogical tree shown in Fig. 1. Analysis of patterns of mutation between ancestors and descendants on this tree shows that natural selection improved the haemoglobin molecule and then preserved the improvements. Early evolution proceeded at a faster rate (mutational replacements per unit time) than later evolution. This can be shown to correlate with Darwinian theory by taking advantage of the identification of functional residues (Table 1) by Perutz and coworkers⁴⁸⁻⁵³. We have deduced that during the evolutionary transition of monomeric haemoglobin to a tetramer with a sigmoid oxygen equilibrium curve the residue positions which acquired cooperative functions changed more rapidly than other positions. Such a pattern cannot be explained by the theory of random fixation of

selectively neutral mutations⁵⁴, so called non-Darwinian evolution⁵⁵. It can be attributed, however, to positive selection for more optimal function.

Reconstruction of genealogy

A maximum parsimony approach^{56,57} was used to reconstruct the genealogy of the globin chains. According to the parsimony principle⁵⁸⁻⁶², the best genealogy requires the fewest possible nucleotide replacements. In such a genealogy the maximum number of sequence similarities is associated with common ancestry, and the minimum number with parallel and back changes.

A mathematically rigorous procedure reconstructs the most parsimonious ancestors for a given genealogy⁵⁶. There is no known method, short of an exhaustive search, for finding the most parsimonious genealogy. Thus we used an heuristic approach, in which an initial, unweighted pair-group cluster analysis⁶³ was improved repeatedly by a branch-swapping procedure⁵⁷. The search was extended by sliding portions of the globin alignment in order to further reduce the length (that is total number of nucleotide replacements) of the genealogy.

Regions in the genealogy in which few nodal points span long periods of time were underrepresented with respect to nucleotide replacements. Thus we compensated for lost

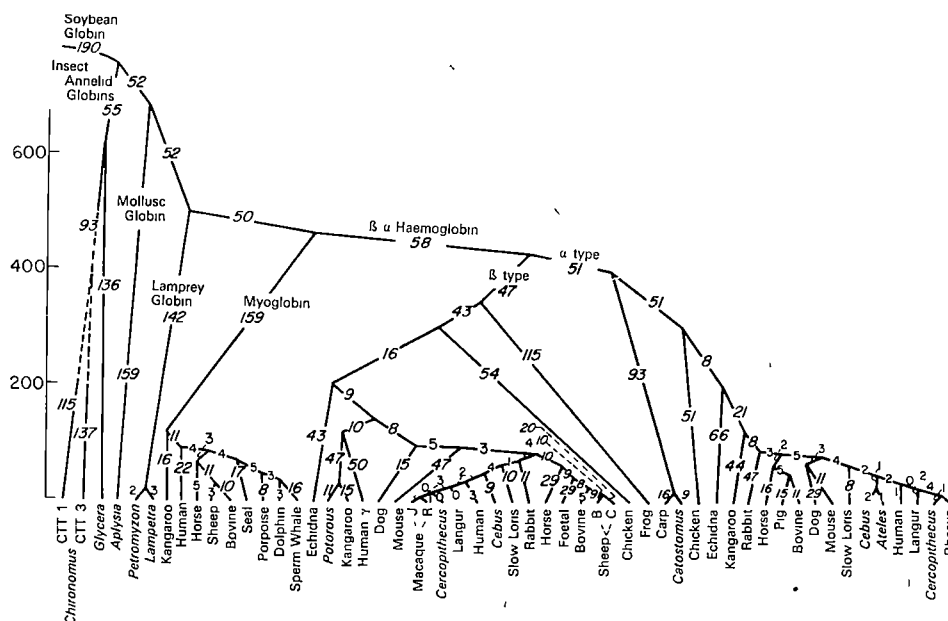


Fig 1 Globin genealogical tree Soybean¹, insect CTT-1 (Braunitzer, unpublished) and CTT-3 (ref 2) (*Chironomus thummi*), annelid (*Glycera dibranchiata*)³, mollusc (*Aplysia limacina*)⁴, *Petromyzon marinus*⁵, *Lampetra fluviatilis*⁶, myoglobin of kangaroo (*Megaleia rufa*)⁷, human⁸, horse⁹, sheep¹⁰, bovine¹¹, harbour seal (*Phoca vitulina*)¹², porpoise (*Phocaena phocaena*)¹³, dolphin (*Delphinus delphis*)¹⁴, sperm whale (*Physeter catodon*)¹⁵, β echidna (*Tachyglossus aculeatus*)¹⁶, β *Potorous tridactylus*¹⁷, β kangaroo (*Macropus giganteus*)¹⁸, γ human¹⁹, β dog²⁰, β mouse C57BL²¹, β Japanese macaque (*Macaca fuscata*)²², β rhesus (*Macaca mulatta*)²³, β *Cercopithecus aethiops*²⁴, β langur (*Presbytis entellus*)²⁵, β human²⁶, β *Cebus apella*²⁷, β slow loris (*Nycticebus coucang*)²⁸, α carp (*Cyprinus carpio*)²⁹, α *Catostomus clarkii*³⁰, α dog³¹, α mouse C57BL³², α slow loris (*Nycticebus coucang*)³³, α *Cebus apella*³⁴, α *Ateles geoffroyi*³⁵, α human³⁶, α langur (*Presbytis entellus*)³⁷, α *Cercopithecus aethiops*³⁸ and α rhesus (*Macaca mulatta*)³⁹. Link lengths are the numbers of nucleotide replacements between adjacent ancestor and descendant sequences and are italicised when our augmentation algorithm corrects for superimposed replacements. Original and corresponding augmented link lengths are as follows: 0-0, 1-1, 2-2, 3-3, 4-4, 5-5, 6-8, 7-9, 8-10, 9-11, 10-15, 11-16, 12-17, 14-20, 15-21, 16-22, 18-29, 20-43, 21-44, 24-47, 27-50, 28-51, 29-52, 31-54, 32-55, 34-58, 36-66, 50-93, 59-115, 63-136, 64-137, 68-142, 85-159, 90-190. The ordinate is a time scale, in Myr based on palaeontological views concerning the ancestral separations of the organisms from which the globins came.

nucleotide replacements in these underrepresented regions by an augmentation procedure⁵⁷ based on the distribution of nucleotide replacements in densely sequenced regions. The results obtained by our algorithm are highly correlated with results obtained by the stochastic method using Poisson distribution^{64,65} for estimating hidden mutational change in codons (G W M, M G, and R Holmquist unpublished).

Myoglobin-haemoglobin separation

In the genealogy and alignments found earlier⁶⁶ the line from the ancestral metazoan globin to the vertebrate β-α haemoglobin ancestor gave off in succession annelid, insect, mollusc, lamprey and mammalian myoglobin branches. Its length was 1,636 nucleotide replacements. Our current genealogy (Fig 1) with its corresponding alignments requires 1,629 replacements. It joins annelid and insect branches, but otherwise retains the same topology. The ancestral separation between mammalian myoglobin and β-α haemoglobin branches still occurs after the lamprey separation. To change the position of the mammalian myoglobin branch costs too many additional mutations. (The aligned sequences of the 55 contemporary globins and the reconstructed codons for these sequences as well as for the 53 ancestral nodes may be obtained by request to the authors.)

Non-uniform globin evolution

The numbers on the links in Fig 1 are the nucleotide replacement values separating adjacent ancestral and descendant sequences. Replacement values corrected for superimposed mutations are italicised. These values were used in Table 2 for calculating the rates of globin evolution during different evolutionary periods. The estimated time scale is based on palaeontological views⁶⁷⁻⁷⁰, some of which have to be considered educated guesses. Perhaps the most uncertainty pertains

to the points for the lamprey-vertebrate and invertebrate-vertebrate dichotomies. The times chosen of 500 Myr ago for the former and 680 Myr ago for the latter are more ancient than actually documented in the fossil record. These dichotomies are, however, not less than 420 and 540 Myr old respectively⁷¹. By the same token, the fossil record of the late Precambrian, while revealing unicellular organisms in abundance, provides no hint of multicellular life until very close to the end of this epoch⁷²⁻⁷⁴. Thus the ancestral separation of invertebrates (molluscs, insects, annelids) and chordates was not likely to have been earlier than 650-700 Myr ago, and it could have been somewhat later.

On *a priori* grounds⁷⁵⁻⁷⁹ and from results of Table 2, we think that rapid rates followed by slow rates occurred in globin evolution—first, when selection shaped the prototypic globin, then when new functions emerged in members of the globin family. The most ancestral portion of the reconstructed genealogy is too sparsely populated to test this hypothesis adequately. Thus Table 2 starts from the invertebrate-vertebrate ancestor at 680 Myr ago, and focuses on the vertebrates. The encompass the denser region of the genealogy and are more readily dated by palaeontological evidence. Rates are 1 nucleotide replacements per 100 codons per 10⁸ yr (NR%). During the span from the late Precambrian to the present, the globin genes evolved at an average rate of 31 NR%. This is more than three times faster than the rates found in studies^{78,80,81} in which distances between the contemporary sequence were not corrected sufficiently for superimposed mutation. Moreover, in contradistinction to conclusions⁸² on constancy of evolutionary rates, the haemoglobin genes evolved at markedly non-constant rates. For the first 380 or so Myr from the invertebrate-vertebrate to the amniote (chicken-mammal) ancestor, the haemoglobin genes evolved at an average rate of 46 NR%, but for the next 300 Myr from the amniote

ancestor to the present they evolved at the average rate of only 15 NR%. Comparable values for the unaugmented lengths are 25 NR% in the earlier period and 11 NR% in the later period. This difference is supported by a χ^2 analysis, which uses the above time estimates and the uniform rate assumption as the null hypothesis (augmented $\chi^2 = 347.3043$, $P < 0.001$, 1 d.f., unaugmented $\chi^2 = 102.8298$, $P < 0.001$, 1 d.f.). Non-uniform globin evolution has also been observed by others⁸³.

As Table 2 shows, globin evolution accelerated in the early vertebrates. This acceleration correlates with gene duplications, which first separated the vertebrate myoglobin and haemoglobin branches and then subdivided the haemoglobin branch into α and β lineages. From 500 to 400 Myr ago the genes descending from the basal vertebrate ancestor through the haemoglobin-myoglobin and β - α ancestors to the teleost-tetrapod α ancestor evolved at the rate of 109 NR%. For the following 100 Myr between the teleost-tetrapod and amniote ancestors, the rate of α evolution slowed to 36 NR%, and then over the next 300 Myr (to the present) to an average rate of 14.5 NR%. When the vertebrate haemoglobin genes are traced from the 500 Myr old vertebrate ancestor to the tetrapod β ancestor of about 340 Myr ago, the rate of nucleotide replacements comes out to 66 NR%. As the rate was 109 NR% for the genes traced to the 400 Myr old teleost-tetrapod α ancestor,

β genes at first must have evolved less rapidly than α genes. Between the tetrapod and amniote β ancestors, the β rate accelerated to 74 NR%, but then β evolution, like α evolution, abruptly slowed. The different lines of β descent from the amniote ancestor to the present evolved at an average rate of only 16 NR%.

Selectionist interpretation

We interpret the initial acceleration-eventual deceleration pattern in protein evolution this way. When mutations first produce a potentially useful protein, the possibilities for improvements are considerable. Thus mutations in the gene coding for the protein will often be advantageous and selected for, but after the protein has been perfected, a much larger proportion of mutations would be detrimental and selected against. Once many proteins have become optimised, that is more intricately coadapted for the cooperative execution of their functions, macromolecular evolution would slow. Such a pattern, however, could be temporarily interrupted by periods of accelerated evolution. This happened in early vertebrates. Mutations were selected in duplicated genes which multiplied the number of proteins with differentiated functions. Accelerated change occurred while selection was optimising the structures of these new proteins, but eventually the stabilising component of selection slowed the further rate of change.

Table 1 Residue positions with defined functional roles in mammalian α and β haemoglobin chains

Helical position	β functions	α functions	Helical position	β functions	α functions
	Haem contact sites			Non-Bohr-associated, $\alpha_1\beta_1$ contact sites	
B13	HC, IP	HC, IP	B11		$\alpha_1\beta_1$
C4	HC, IP	HC, IP	B12		$\alpha_1\beta_1$
C7	HC	HC, $\alpha_1\beta_2$	B15	$\alpha_1\beta_1$	$\alpha_1\beta_1$
CD1	HC, IP	HC, IP	B16	$\alpha_1\beta_1$	$\alpha_1\beta_1$
CD3	HC	HC	C1		$\alpha_1\beta_1$
CD4	HC, IP	HC, IP	D2	$\alpha_1\beta_1$	
E7	HC	HC	D6	$\alpha_1\beta_1$	
E10	HC		G10	$\alpha_1\beta_1$	$\alpha_1\beta_1$
E11	HC, IP	HC, IP	G11		$\alpha_1\beta_1$, IP
E14	HC		G13		$\alpha_1\beta_1$
E15	HC, IP		G14	$\alpha_1\beta_1$	$\alpha_1\beta_1$
F4	HC	HC	G17	$\alpha_1\beta_1$	
F7	HC	HC	G18	$\alpha_1\beta_1$	$\alpha_1\beta_1$
F8	HC	HC	GH2	$\alpha_1\beta_1$	$\alpha_1\beta_1$
FG3	HC	HC, $\alpha_1\beta_2$	GH5	$\alpha_1\beta_1$	$\alpha_1\beta_1$
FG5	HC, $\alpha_1\beta_2$, IP	HC, $\alpha_1\beta_2$, IP	H1	$\alpha_1\beta_1$	
G4	HC, $\alpha_1\beta_2$	HC	H2	$\alpha_1\beta_1$	$\alpha_1\beta_1$
G5	HC, IP	HC, IP	H3	$\alpha_1\beta_1$	
G8	HC, IP	HC, IP	H5	$\alpha_1\beta_1$	
H12		HC, IP	H6	$\alpha_1\beta_1$	$\alpha_1\beta_1$
H15	HC, IP	HC, IP	H9	$\alpha_1\beta_1$	
H19	HC, IP	HC, IP			
	Non-haem, $\alpha_1\beta_2$ contact sites			Remaining interior positions (stabilises tertiary structure)	
C2	$\alpha_1\beta_2$	$\alpha_1\beta_2$	A8	IP	IP
C3	$\alpha_1\beta_2$	$\alpha_1\beta_2$	A11	IP	IP
C5	$\alpha_1\beta_2$	$\alpha_1\beta_2$, BE	A12	IP	IP
C6	$\alpha_1\beta_2$	$\alpha_1\beta_2$	A15	IP	IP
CD2	$\alpha_1\beta_2$	$\alpha_1\beta_2$	B6	IP	IP
FG4	$\alpha_1\beta_2$	$\alpha_1\beta_2$	B9	IP	IP
G1	$\alpha_1\beta_2$	$\alpha_1\beta_2$	B10	IP	IP
G2	$\alpha_1\beta_2$	$\alpha_1\beta_2$	B14	IP	IP
G3	$\alpha_1\beta_2$	$\alpha_1\beta_2$	D5	IP	
HC2	$\alpha_1\beta_2$, IBE, IP	$\alpha_1\beta_2$, IBE, IP	E4	IP	IP
HC3	$\alpha_1\beta_2$, $\beta\beta$, BE		E8	IP	IP
Non- $\alpha_1\beta_2$, like-chain contact, Bohr effect, and 2,3-DPG binding sites			E12	IP	IP
NA1	$\beta\beta$, DPG	$\alpha\alpha$, BE	E15		IP
NA2	$\beta\beta$, DPG		E18	IP	IP
A4		$\alpha\alpha$, IBE	E19	IP	IP
B12	IBE, $\alpha_1\beta_1$		F1	IP	IP
C1	IBE, $\alpha_1\beta_1$		G11	IP	
EF6	DPG		G12	IP	IP
FG1	BE		G16	IP	IP
G6		$\alpha\alpha$	H8	IP	IP
H5		IBE, $\alpha_1\beta_1$	H11	IP	IP
H9		$\alpha\alpha$, BE, $\alpha_1\beta_1$	H12	IP	
H10	$\beta\beta$	$\alpha\alpha$, IBE			
H21	$\beta\beta$, DPG				
HC3		$\alpha\alpha$, BE			

HC, Haem contact, IP, interior position, $\alpha_1\beta_2$, $\alpha_1\beta_2$ contact, BE, Bohr effect site, IBE, interacts with Bohr effect site, $\beta\beta$, $\beta\beta$ contact, $\alpha\alpha$, $\alpha\alpha$ contact, DPG, 2,3-diphosphoglycerate binding site, and $\alpha_1\beta_1$, $\alpha_1\beta_1$ contact

We assume that homotetramers preceded heterotetramers in haemoglobin evolution. This would explain why the amino acids involved in interchain contacts occur in α and β chains mostly at homologous residue positions (Table 1). We assume further that the ancestral homotetramer had subunits more β -like in polymer forming properties than α -like, because homotetramers are readily formed by β chains but not by α ^{84,85}. Many invertebrate haemoglobins are monomers. Lamprey haemoglobin chains in the oxygenated state are also monomers. But in the deoxy state, they form homodimers⁸⁶ and transitory tetramers⁸⁷. Thus a haemoglobin more complicated than a simple monomer probably existed in the earliest vertebrates, as lampreys belong to the most primitive vertebrate class. This first vertebrate haemoglobin, if it behaved like lamprey haemoglobin, released oxygen from its haem iron atoms more readily at reduced pH and in its aggregated state. It would have been, therefore, a more useful protein than the homotetramers of β chains which lack even the rudiments of cooperativity^{84,85}. Natural selection could then exploit the possibilities for bringing about a haemoglobin more efficient at delivering oxygen to the tissues of larger-bodied, fast-moving animals. This required the evolution of heterotetrameric haemoglobin.

After the β - α gene duplication in a common ancestor of teleosts and tetrapods, positive Darwinian selection acted on the nascent α locus while stabilising selection was stronger at the β locus. Once the older β_4 type homotetramer was, however, replaced by the heterotetramer with a specialised α chain, positive selection for a more differentiated β chain intensified. This caused the rate of β evolution to accelerate between the tetrapod and amniote ancestors. Functionally superior tetrameric haemoglobin emerged. Then α and β loci were both subjected to intense stabilising selection. Evidence for this selectionist interpretation is provided by the following more detailed analysis.

Positive selection for improved function

The residue positions in Table 1 are arranged according to their functions in present day mammalian α and β chains. The positions with the most crucial functions are first the haem contacts and second those concerned with the interchain

cooperativity which facilitates oxygen delivery, the $\alpha_1\beta_2$ contacts and the salt-bridge-forming sites associated with the Bohr effect, respectively. There are many nucleotide replacements between the mollusc-vertebrate globin and amniote α and β ancestors (Table 3) at these positions, but almost no changes in the amniotes (Tables 4 and 5).

Table 3 shows the replacement values from the invertebrate-vertebrate ancestor through the basal vertebrate and the myoglobin-haemoglobin and β - α ancestors to the basal amniote α and β sequences. The most slowly evolving positions are those which now function in both α and β haemoglobin chains as haem contacts, and which must have been well established as such as far back as 600–700 Myr ago. Yet these haem contacts, although conservative compared with other residue positions, show a seven times faster evolutionary rate in the preamniotes than in the amniotes (compare Tables 3, 4, and 5). Presumably, haem-haem cooperative interactions were not fully perfected until about 300 Myr ago.

Positive Darwinian selection in the emerging vertebrates is indicated by the high proportion of changes at positions which now function as $\alpha_1\beta_2$ contacts in both α and β chains. These contacts favour haem-haem interactions by placing the haems of the contacting chains in close proximity. Positions which acquired this function evolved four times faster than the average rate over all positions (Table 3). Mutations occurred at C5, C6, FG4, G1 and HC2, five of the nine positions which now function in both α and β chains as $\alpha_1\beta_2$ contacts, as well as at FG3, a haem contact which also functions as an $\alpha_1\beta_2$ contact in amniote α chains. The mutations of glutamic acid (GAG) to glutamine (CAG) at C5, valine (GUG) to glutamine (CAG) at FG4, asparagine (AAU) to aspartic acid (GAU) at G1, isoleucine (AUU) to tyrosine (UAU) at HC2, and arginine (CGU) to leucine (CUU) at FG3 persisted. They are central to the interchain cooperativity of present-day tetrameric haemoglobin.

Between the myoglobin-haemoglobin and β - α ancestors, the sites which now function as $\alpha_1\beta_1$ contacts in both α and β chains evolved at twice the average rate of all positions (Table 3). Lysine (AAA) mutated to isoleucine (AUA) at B15, aspartic acid (GAU) to asparagine (AAU) at G10, aspartic acid (GAU)

Table 2 Rates of mollusc and vertebrate globin evolution

Evolutionary period	Age (Myr BP) Mollusc, agnathan, and jawed vertebrate globins	Nucleotide replacements per 100 codons per 10 ⁸ yr
Invertebrate-vertebrate ancestor to <i>Aplysia</i> 'myoglobin'	680– 0	16.1
Invertebrate-vertebrate ancestor to vertebrate globins	680– 0	31.2 (20.5–35.3)
Invertebrate-vertebrate to vertebrate ancestor	680–500	19.5
Vertebrate ancestor to lamprey globins	500– 0	21.0 (20.9–21.0)
Vertebrate ancestor to myoglobins	500– 0	31.6 (29.6–33.2)
Vertebrate ancestor to α_s and β_s	500– 0	37.1 (33.5–40.9)
Vertebrate to therian myoglobin ancestor	500–120	36.2
Therian myoglobin ancestor to myoglobins	120– 0	17.2 (8.7–23.4)
Vertebrate to tetrapod β ancestor	500–340	65.7
Tetrapod β ancestor to β_s	340– 0	22.9 (18.3–28.4)
Vertebrate to teleost-tetrapod α ancestor	500–400	108.9
Teleost-tetrapod α ancestor to α_s	400– 0	19.9 (18.1–23.9)
α Lineage		
Teleost-tetrapod ancestor to teleost α_s	400– 0	18.7 (18.1–19.3)
Teleost-tetrapod ancestor to amniote α_s	400– 0	20.0 (18.6–23.9)
Teleost-tetrapod to amniote α ancestor	400–300	36.2
Amniote ancestor to chicken α	300– 0	12.1
Amniote ancestor to mammalian α_s	300– 0	14.7 (12.5–19.9)
Amniote to eutherian α ancestor	300–90	12.5
Eutherian ancestor to human α	90– 0	13.4
Eutherian ancestor to all eutherian α_s	90– 0	18.5 (12.6–37.0)
β Lineage		
Tetrapod ancestor to frog β	340– 0	24.2
Tetrapod ancestor to amniote β_s	340– 0	22.8 (18.3–28.4)
Tetrapod to amniote β ancestor	340–300	73.6
Amniote ancestor to chicken β	300– 0	12.3
Amniote ancestor to mammalian β_s	300– 0	16.3 (12.3–22.3)
Amniote to eutherian β ancestor	300–90	10.8
Eutherian ancestor to human β	90– 0	16.7
Eutherian ancestor to all eutherian β_s	90– 0	26.6 (11.4–49.4)

Table 3 Nucleotide replacement lengths for groups of residue positions

Type of residue positions	During emergence of hypothetical homopolymer						During evolution of nascent heterotetramer			
	Invert-vert to vert anc		Vert to myo-Hb anc		Myo-Hb to β - α anc		β - α to amniote α anc		β - α to amniote β anc	
	No of positions	Average length	No of positions	Average length	No of positions	Average length	No of positions	Average length	No of positions	Average length
Haem contacts in both β and α and in myoglobin chains	18	0.11	18	0.17	18	0.06	18	0.17	18	0.11
Non-haem $\alpha_1\beta_2$ contacts in both β and α chains	9	0.78	9	0.00	9	0.22	9	0.67	9	0.44
Like-chain contacts in both β and α chains	3	0.00	3	0.00	3	0.00	3	0.33	3	0.67
$\alpha_1\beta_1$ contacts in both β and α chains	13	0.23	13	0.23	13	0.38	13	0.31	13	0.31
With defined functions in α but not β chains	7	0.14	7	0.14	7	0.29	7	0.86	7	0.14
With defined functions in β but not α chains	12	0.33	12	0.08	12	0.17	9	0.11	12	0.67
Remaining interior positions	19	0.16	19	0.26	19	0.05	18	0.22	19	0.26
Remaining exterior positions	67	0.13	67	0.21	67	0.31	64.5	0.48	65	0.28
All positions	148	0.20	148	0.18	148	0.23	141.5	0.40	146	0.30

Positions are grouped according to their functional roles in present-day haemoglobin chains, as may be identified from Table 1, unaugmented nucleotide replacements for the positions in each group during each period of descent are averaged. B12, C1, H5, and H9 are included in the group consisting of $\alpha_1\beta_1$ contacts in both β and α chains since the emergence of this function probably preceded the emergence of their Bohr-effect-related function. The seven positions with defined functions in α but not β chains are A4, B11, CD2, G6, G11, G13, and H12.

The twelve positions with defined functions in β but not α chains are NA2, D2, D6, E10, E14, E15, EF6, FG1, G17, H1, H3 and H21.

to valine (GUU) at G14, alanine (GCG) to proline (CCG) at GH2, and also alanine (GCU) to proline (CCU) at H2. Then these amino acids, along with those at other prospective $\alpha_1\beta_1$ contact sites, either persisted or mutated to similar amino acids in later amniote haemoglobin chains. This supports the notion that the β - α gene was coding for homotetrameric haemoglobin even before it duplicated. Conversely in the myoglobin lineage, tyrosine mutated to histidine, tyrosine to lysine, leucine to histidine, aspartic acid to proline, proline to valine, and glutamic acid to lysine at C1, C7, FG3, G1, G2, and H9, respectively. These mutations at positions which function as interchain contacts in haemoglobin polymers helped reshape the myoglobin molecule into a high oxygen affinity monomer.

The positions which are $\alpha_1\beta_2$ contacts in the two haemoglobin chain types evolved at a faster than average rate in the nascent α and β lineages (Table 3). Also the positions categorised as like-chain contacts in both α and β chains evolved at a faster than average rate because of mutations at HC3, a Bohr effect site in both α and β chains, as well as an $\alpha_1\beta_2$ contact site in β chains. (Ancestral lysine (AAG) mutated to arginine (AGG) at α HC3 and to histidine (CAU) at β HC3). Positions which acquired important functions in α chains but not in β evolved in the α lineage at a faster rate than other positions. Similarly, positions which acquired important functions in β chains but not in α evolved in the β lineage at a faster rate than other positions.

The evidence in Table 3 for positive selection directing the evolution of early vertebrate haemoglobin is supported by a χ^2 test, performed to test the null hypothesis that sites where mutations produced acquired crucial functions did not evolve at a different rate from all other sites. Homopolymer emergence was tested by comparing nucleotide replacements at prospective $\alpha_1\beta_2$ and $\alpha_1\beta_1$ contact positions with all other positions in the invertebrate-vertebrate to vertebrate and myoglobin-haemoglobin to β - α ancestors ($\chi^2 = 7.312$, $P < 0.01$, 1 d.f.). Heterotetramer evolution was tested by comparing nucleotide replacements at $\alpha_1\beta_2$ contacts, like-chain contacts, and α -only functions in the β - α to amniote α lineage, plus $\alpha_1\beta_2$ contacts, like-chain contacts, and β -only functions in the β - α to amniote β lineage, with the other positions ($\chi^2 = 11.403$, $P < 0.001$, 1 d.f.).

Mutations were fixed in the diverging α and β genes because they produced functionally superior haemoglobin molecules. By the time amniote tetrapods evolved, heterotetramers with modern allosteric properties had replaced the older models.

Thereafter natural selection acted against further amino acid substitutions at the residue positions involved in haem and $\alpha_1\beta_2$ contacts and the Bohr effect. Nucleotide replacements occurred in the codons for these positions at only one-fifth to one-tenth the average rate (Tables 4 and 5). Moreover, the average rate itself was much reduced.

Darwinian selection, however, was still directing haemoglobin evolution. This is demonstrated by nucleotide replacements at 2,3-diphosphoglycerate (DPG)-binding sites. For example, between the amniote and mammal (protherian and therian) β ancestor, glutamine (CAG) at H21 mutated to histidine (CAU). The greater binding of DPG to the β chain produced by this mutation further facilitated the unloading of oxygen from haemoglobin in the deoxy conformation^{51,53}. The effect was reversed in the line to human γ chain by H21 histidine mutating to serine. This mutation favoured the ability of foetal haemoglobin to compete with adult haemoglobin for oxygen.

Darwinian selection can also explain changes observed at positions NA2, A2, A7, CD2, D2, E2, EF5, G18, GH2 and H2 in descent from the metazoan ancestor to human α and β sequences, proline replaced alanine five times more often than alanine replaced proline. Alanines can appear anywhere in a globin chain, but prolines are restricted to sites at the end of helices or to non-helical regions where they delimit the lengths.

Table 4 Effect of functions on nucleotide replacement lengths in the amniote ancestor-to-contemporary α lineages

Type of residue positions	No of positions	Average length	Nucleotide replacements per position per 100 Myr
Haem contacts	19	0.37	0.02
Non-haem contact $\alpha_1\beta_2$ contacts	10	0.20	0.01
Salt bridges, associated Bohr effect, $\alpha\alpha$ contact	7	0.14	0.01
Non-salt bridge $\alpha_1\beta_1$ contacts	14	2.00	0.13
Remaining interior positions	19	1.47	0.09
Remaining exterior positions	72	2.38	0.15
All positions	141	1.68	0.11

Positions are grouped according to their functional roles, as may be identified in Table 1, unaugmented nucleotide replacements over the amniote (chicken-mammal) α region of the globin genealogy are averaged for the positions in each group. The times of the connecting links over the amniote region, when added up, represent about 1.6×10^9 yr.

Table 5 Effect of functions on nucleotide replacement lengths in the amniote ancestor-to-contemporary β lineages

Type of residue positions	No of positions	Average length	Nucleotide replacements per position per 100 Myr
Haem contacts	21	0.43	0.02
Non-haem contact $\alpha_1\beta_2$ contacts	10	0.40	0.02
Salt bridges, associated Bohr effect, $\beta\beta$ contact	4	0.00	0.00
2,3-DPG binding	4	1.75	0.10
Non-salt bridge $\alpha_1\beta_1$ contacts	16	2.81	0.16
Remaining interior positions	21	1.57	0.09
Remaining exterior positions	70	3.34	0.20
All positions	146	2.27	0.13

Positions are grouped according to their functional roles, as may be identified in Table 1, unaugmented nucleotide replacements over the amniote (chicken-mammal) β region of the globin genealogy are averaged for the positions in each group. The times of the connecting links over the amniote β region, when added up, represent about 1.8×10^9 yr.

of helical segments⁴⁸. An alanine to proline mutation at most positions would be detrimental but at certain positions would be advantageous by producing a more stably directed helix. The greater incidence of proline at such positions in human haemoglobin than in ancestral primitive haemoglobins may be attributed to positive selection bringing about further improvements.

Continuing Darwinian evolution

The positions in amniote haemoglobins which have undergone the most evolutionary change are those surface or exterior positions without sharply defined functions (Tables 4 and 5). This apparently supports the neutralist argument that most amino acid substitutions can be accounted for by mutations which escape the scrutiny of natural selection⁸². These surface changes, however, can also be interpreted on selectionist grounds. Having fixed the features in the haemoglobin tetramer responsible for its crucial (and more obvious) functions, natural selection would then be able to shape the finer adaptations of the protein. These adaptations should especially involve the exterior sites, because of their potential for interacting with other macromolecules. Amino acid changes in these exterior positions can be viewed as further steps in an optimisation process in which the functions of proteins become more effectively coordinated. Evolutionary change at the molecular level must correspond in some way to the genotypes of whole organisms. Since the net balance of many gene actions guides organismal selection⁸⁸, the finer coadaptations among proteins must await the coarser adaptations. While these are being selected a proportion of amino acid substitutions, at the less critical sites, should indeed fit the neutral mutation model. Positive organismal selection, however, eventually improves the subtler protein interactions. Positive and then stabilising selection might well explain the rapid rate of haemoglobin evolution in the early placental mammals and the later greatly diminished rate in hominoids.

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letters to nature

Cosmic rays and the Galaxy

EVIDENCE on whether the majority of detected cosmic rays originate in our Galaxy has been inconclusive arguments based on the abundance of uranium nuclei, the power available in supernovae, γ -ray emission, the absence of a 10^{20} eV cutoff, and the galactic origin of the electron component all involve uncertainties, but in each case a galactic origin seems to offer a more probable interpretation than an extragalactic origin. Near isotropy has until now been the discordant feature. Work by Krasilnikov *et al.*¹ and Bell *et al.*² now suggests that the arrival directions of the most energetic cosmic rays are almost certainly highly anisotropic. Although the distribution of arrival directions is quite different from that predicted by Karakula *et al.*³ for particles from galactic sources, we point out here that the evidence now indicates strongly a galactic origin for even the most energetic cosmic rays that reach us, we also suggest that there is a very extensive ordered magnetic field outside the plane of our Galaxy.

A 'local' (that is, galactic) magnetic field which is incapable of completely trapping locally produced particles cannot introduce anisotropy into radiation that originated in very distant parts of the Universe, and is therefore supposedly isotropic, so it is necessary to seek a localised source essentially in the Virgo Supercluster or in our Galaxy. Figure 1 shows the distribution of arrival directions of 84 showers above 2×10^{19} eV (from ref. 4). They are plotted in celestial coordinates (Fig. 1a) and the distribution in galactic longitude of showers north or south of the galactic plane is also shown (Fig. 1b). Another 20 showers of almost this energy are included (Fig. 1a) as small points (D. D. Krasilnikov, unpublished), they reinforce the statistical significance of the anisotropy¹, but they are ignored in (Fig. 1b), as without them the data represent an almost uniform exposure to all parts of the sky.

The RA distributions in the north and south hemispheres have significant first and second harmonics respectively, of amplitude $>60\%$, associated with clustering at L, M, N (see Fig. 1a). The existence of widely-spaced maxima, and the wide spread of the most energetic particles do not suggest propagation from a single distant source such as the cluster centre in Virgo. If the supercluster is equivalent to 3,000–10,000 galaxies like our own, but at 18 Mpc as against 9 kpc, the inverse square law alone gives our Galaxy an advantage of more than 400 even if it does not trap cosmic rays, that is, unless it is a weaker than average source. At GeV energies it is not, as synchrotron radiation from our Galaxy and from the possible weak belt of emission from the supercluster⁵ are in about this ratio, and the strong sources Virgo A and Centaurus A (see Fig. 1a) do not change the balance significantly¹². Moreover, the cosmic-ray energy spectrum to the highest energies⁶ now seems essentially continuous with that at GeV energies, in spite of earlier estimates⁷.

Three aspects of the observed anisotropies accord well with a galactic origin, however, when observations at all energies are considered. First, the time of maximum intensity, which in the northern hemisphere switches from ~ 19 h sidereal time below 4×10^{15} eV ($=E_0$), to ~ 13 h above this energy⁸, is still near 13 h at 5×10^{18} – 10^{20} eV (ref. 1). This suggests that one mode of propagation below E_0 , consistent with an escape along magnetic field lines, is overtaken above E_0 by a more rapid drift, perhaps radially outwards across field lines, bringing maxima more normal to

the galactic plane⁹. This motion could persist to the highest energies.

Second, above E_0 , the cosmic-ray flux does indeed fall more steeply, and if it is supposed that the galactic sources inject particles at the rate $Q(E)dE \propto E^{-2.5}dE$ (say), one has an intensity modified by the falling lifetime, $T(E)$, of retention in the Galaxy $J(E) \propto Q(E)T(E)$. Thus, if the anisotropy, A , varies inversely as trapping lifetime, as should be approximately true, $A(E) \propto Q(E)/J(E)$. In Figure 2, the line $E^{-2.5}/J(E)$ (from ref. 6) is superimposed on a plot of anisotropy measurements, with selected data of high statistical weight^{1,2,10}, an average of many results near 10^{17} eV, and harmonic amplitudes present in other tables^{7,11}. The energy dependence of anisotropy, and the form of the energy spectrum, can be seen together to suggest an explanation in terms of more rapid escape from the Galaxy above E_0 .

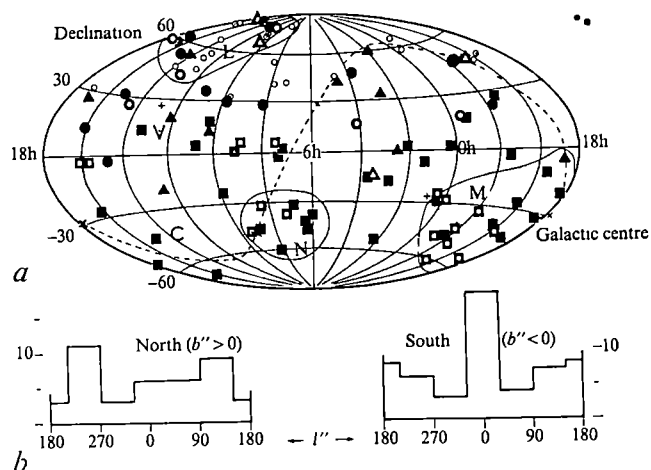


Fig. 1 a, Arrival directions of 84 showers above 2×10^{19} eV. ■, Sydney, ▲, Volcano Ranch, ●, Haverah Park. The most energetic 30% are shown as open symbols. Twenty showers above 10^{19} eV, ○, Yakutsk. Plotted in coordinates of right ascension (increasing to left, with 6 h in centre) and declination. The galactic plane is shown dashed, galactic poles as crosses. The dotted line joins the south galactic pole to the galactic centre. b, Distribution of the 84 showers in galactic longitude, plotted separately for north and south galactic latitudes. V, Virgo A, C, Centaurus A, L, M, N, clustering.

Third, returning to the highest energies, the maximum M , the most prominent in the harmonic analysis, which is responsible for the peak in Fig. 1b (south), is centred on galactic longitude 0° (Fig. 1a). It lies south of the region of strong synchrotron and gamma-ray emission, as though deflected by a large-scale magnetic field directed towards galactic longitude 270° to the south of the plane, between us and the centre of the Galaxy. The group N (Fig. 1a) may be accidental, but lies roughly in the Vela direction, along the galactic field. The unexpected³ absence of any concentration in the 90° direction, may mean that of the few sources effective at any one time in this energy range, none lies in that direction. But why peak L is displaced in galactic longitude is not clear.

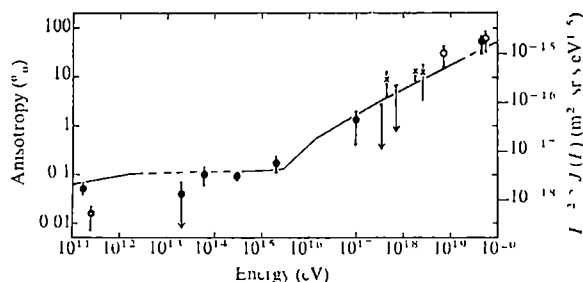


Fig 2 Anisotropy in the right ascension distribution of cosmic rays of different energies, E , along a broad belt of constant declination. The larger of the first (\bullet) or second (\circ) harmonic is plotted \times . Unpublished harmonics present in tables of shower distributions, but only showers in one hemisphere (positive or negative declination) are included. The line is the inverse of the cosmic-ray energy spectrum, multiplied by $E^{-2.5}$.

To retain nuclei of $\sim 10^{20}$ eV would require an ordered magnetic field extending several kpc from the galactic plane, a $2 \mu\text{G}$ field could retain oxygen nuclei, but not lighter nuclei. There is independent evidence, from the extension of observed synchrotron radiation to 1 kpc, that the scale of decay of the magnetic fields must exceed 1 kpc.

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Measured offset between the Crab pulsar and Tau X-1

WE have used the MSSL X-ray detector on board the Copernicus spacecraft to measure the effective diameter of the Crab Nebula and to measure the location of the centroid of the emission with respect to the accurately known pulsar position¹.

On October 7, 1974, the orbit of the satellite took it through the Tau X-1 lunar occultation 'shadow' on two successive orbits, enabling us to observe two disappearances (D_1 and D_2) and two reappearances (R_1 and R_2) of the Crab Nebula with a collimated proportional counter operating in the energy range 2.5-7.5 keV. All four occultations occurred partially during the dead time of the spacecraft data handling system (23.6 s out of every 86.5 s), though R_1 started so close to the end of a live period as to make the data statistically less reliable than the data from the other events.

Figures 1 and 2 show the counts from the detector in successive 3.3-s time intervals during the occultations. No background has been subtracted, the low level of background which is apparent when the source is occulted does not vary by more than 10% during each event, as monitored by the guard counter associated with this detector².

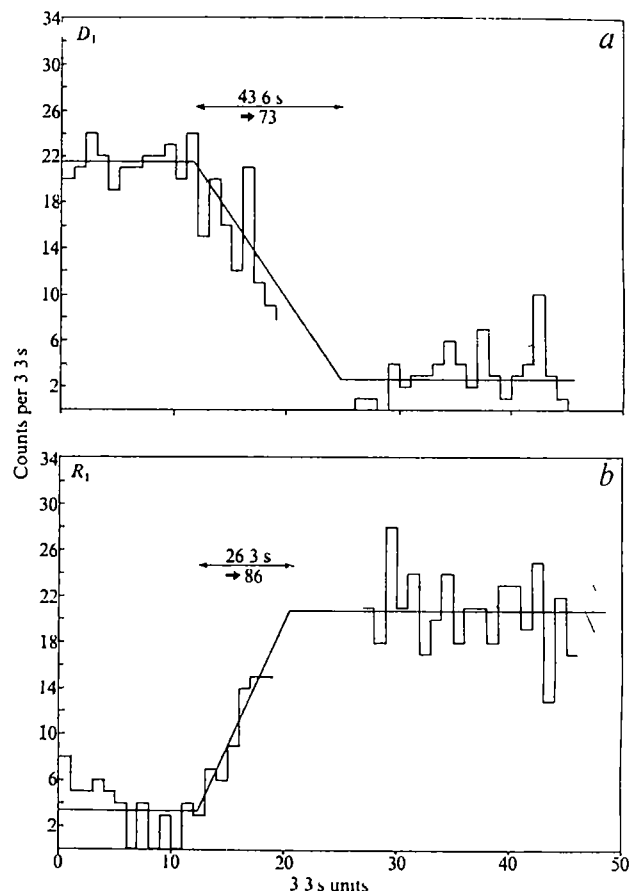


Fig 1 2.5-7.5 keV count rate during first disappearance (a) and reappearance (b). Background has not been subtracted. The solid lines show the best fit to the data. Orbit 11244, $\alpha = 236^\circ$.

The background level does vary from occultation to occultation because of the changing geographical location of the satellite. Figures 1 and 2 also show the best fitting constant count rate before and after each occultation and a linear change in count rate during the course of the occultation. The data do not justify a more complex description. The best fit is defined as that combination of the time of change of slope (t_0) and the value of slope (S) which gives a minimum value of χ^2 when compared with the data. Values of χ^2 were computed for a range of values of t_0 and S and the resultant χ^2 grid was examined to find the minimum. The uncertainty in centroid location was derived by finding the uncertainty in t_0 when S was held fixed at its most probable value, the one sigma uncertainty in source diameter was derived from the maximum and minimum values of S reached by the $(\chi^2_{\text{min}} + 1)$ contour. It is normally recommended³ that interval widths in χ^2 tests should be wide enough to include a minimum of five events each. We have therefore summed the count rate in 3.3-s bins, rather than use our maximum time resolution of 1.65-s, in order to compute valid estimates of χ^2 for each trial fit. Such a summation still leaves R_1 with only two intervals containing more than five events, and so we cannot obtain valid χ^2 values for these data. We therefore restrict discussion to the other three occultations.

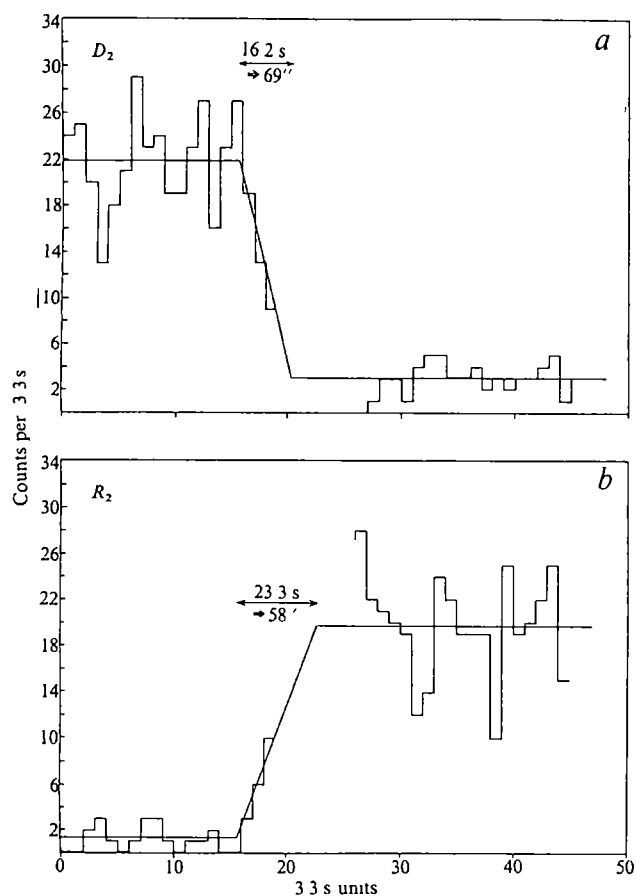


Fig 2 2.5–7.5 keV count rate during second disappearance (a) and reappearance (b). Background has not been subtracted. The solid lines show the best fit to the data. Orbit 11245, $p_a = 273^\circ$.

We used the best estimates of S and t_b to define the effective times at which the occultations started and finished. The apparent position of the Moon's limb relative to the pulsar at these times was calculated using satellite positions derived by the Goddard Space Flight Center tracking group and the lunar ephemeris currently in use at the Royal Greenwich Observatory. We have neglected the irregularities and curvature of the lunar limb.

Table 1 gives the measured effective diameters together with the uncertainties in diameter and in the location of the centroid (measured along the line perpendicular to the lunar limb). A weighted average for R_1 and R_2 is given, as the position angles for these events are within 5° of one another. Figure 3 shows these diameters and centroid locations relative to the pulsar position.

Most of the X-ray flux is asymmetrically disposed with respect to the pulsar. A similar result is suggested by W. Lewin (private communication) from a recent balloon flight. Our effective diameter of $73''$ at position angle (p_a) 236° is similar to the balloon measurement ($60'' \pm 10''$) at p_a 244° , though our value of $71''$ at p_a 275° is substantially larger than the value of Ricker *et al.* of $30'' \pm 10''$ at p_a 130° ($\approx 310^\circ$). The fact that the angle between our occultation passes was less than that in the balloon measurements (39° compared with 66°) would produce some sort of averaging, but not sufficient to account for the whole difference.

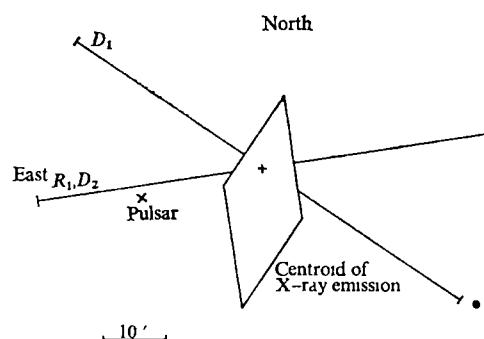
With the exception of the 1964 occultation experiment of Bowyer *et al.*, all previous estimates for the X-ray diameter of the Crab Nebula have measured some form of effective diameter, using several different techniques. Our estimates of $73''$ and $71''$ are in reasonable agreement with the figure of $100''$ from a modulation collimator experiment⁶, $66''$

Table 1 Measured effective diameters

Event	Diameter	1 σ Error	Uncertainty in centroid location (1 σ)	Position angle
D_1	$73''$	$+38''$ $-38''$	$+8''$ $-4''$	236°
R_1, D_2	$71''$	$+15''$ $-8''$	$+4''$ $-7''$	275°

from a 25–100 keV modulation collimator experiment⁷ and $83''$ from a raster scan with the arc minute angular response detectors on the Copernicus satellite⁸. As was clear from these earlier experiments, the X-ray emitting region is significantly smaller than the optical nebula, though not as small as would be expected if models such as that of Wilson⁹ were fully representative of the situation. There also seems to be a lack of any dependence of the X-ray size on photon energy, as may be judged from the basic similarity of the balloon energy results^{4,7} to the lower energy rocket or satellite results. Although Wilson's model could be forced to predict a size comparable to that observed by allowing the relativistic electrons to originate in an injection region of diameter $20''$ rather than in a point source, the model still requires a decrease of angular diameter by some 45% in going from 3 to 30 keV effective measurement energy. This is clearly at variance with the observations. An alternative solution suggested by Wilson was the possibility that the electrons which radiate at X-ray energies have a larger diffusion coefficient. The particular model described was the "magnetic cloud" model (ref 10, p 170) which leads to a predicted 75% increase in diameter in going from 3 to 30 keV. This too is at variance with the observations. It is possible that the electron injection region could still be of significant angular diameter, and that the energy dependence of size of the X-ray emitting region could somehow be suppressed, but in view of the significant displacement between the emission centroid and the pulsar, which is generally presumed to be the ultimate source of the electrons, one must search for some reason why these electrons should generate X-rays predominantly in the region to the west or north-west of the pulsar. Rees and Gunn have suggested¹¹ that the electrons are carried outwards by an expanding magnetic field. If this is so, the observed offset between the pulsar and the X-ray emission region requires either the magnetic field to be expanding from a point not coincident with the pulsar (a second electron injection source in addition to the pulsar?), or the presence of substantial amounts of nebular matter to the south-east of the pulsar which

Fig 3 Our measured diameter along position angle 236° (D_1) and 275° (average of D_2 and R_1). The diamond shape is the area common to the 1σ error bands of the centre of each 'diameter'. The offset from the pulsar position is clearly seen.



would prevent expansion in that direction. In this context, it may be significant that photographs of the filamentary structure of the Crab¹² show a toroidal filament about 1' in diameter just to the north-west of the pulsar, whilst the peak of the optical continuum¹² is located at approximately the same position angle and distance from the pulsar as is our measured centroid.

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Ultraviolet emission lines in the spectrum of Procyon

THE Princeton instrumentation¹ on the satellite Copernicus has been used to observe the F5 IV star, α CMi (Procyon). In addition to previously observed² lines of Mg II, the Lyman- α line of H I (1,216 Å), and the resonance lines of Si III (1,206 Å) and O VI (1,032 Å) have been observed for the first time in an F-type star. Figure 1 shows the observed lines, all of which exceed the two standard deviation level.

The observed Lyman- α profile clearly contains a component due to absorption by interstellar atomic hydrogen in the line of sight to Procyon. It is possible to calculate the range of interstellar hydrogen column densities N_H that would lead to the observed profile, by using the equation for the optical depth in the damping wing of Lyman α , that is $\tau(\lambda) = 9.25 \times 10^{-20} (\lambda - \lambda_0)^{-2} N_H$, where λ_0 is the wavelength of the line centre in Angstroms. Taking the attenuation at each point in the line to be $\exp(-\tau(\lambda))$ gives

$$1.5 \times 10^{17} \text{ cm}^{-2} \lesssim N_H \lesssim 3.0 \times 10^{17} \text{ cm}^{-2}$$

Since the distance³ to Procyon is 3.5 pc the mean density of atomic hydrogen, n_H , lies in the range $0.015 \text{ cm}^{-3} \lesssim n_H < 0.030 \text{ cm}^{-3}$, which is much lower than the value of 0.3 cm^{-3} deduced^{4,5} from observations of early type stars at distances of ~ 100 pc. A similar low value of $n_H = 0.015 \text{ cm}^{-3}$ has been calculated⁶ from the Lyman- α absorption profile in the K star α Boo (Arcturus) which is 11 pc away. So it seems that the neutral hydrogen density in the solar neighbourhood (say to ~ 10 pc) is much lower than the average value out to ~ 100 pc. The low value derived implies that the opacity of the nearby interstellar medium at $\lambda < 912$ Å (the H I Lyman limit) is considerably less than previously assumed and even at 10 pc there should be

about 10% transmission at the Lyman edge and greater than 50% transmission below the He I limit (504 Å). The possibility of making observations in this spectral region in nearby stars is thus limited solely by the intrinsic stellar emission and not by interstellar absorption.

The observed emission line fluxes have been converted to absolute energy fluxes at Procyon using the known angular diameter of the star⁷. Using methods established for analysing the solar extreme ultraviolet spectrum⁸ these fluxes can be converted to the emission measure, $\int n_e n_p^2 dh$, for the temperature region where each line is formed. Figure 2 shows the emission measures observed and the mean solar distribution⁹. A range of possible values is indicated for Si III since the

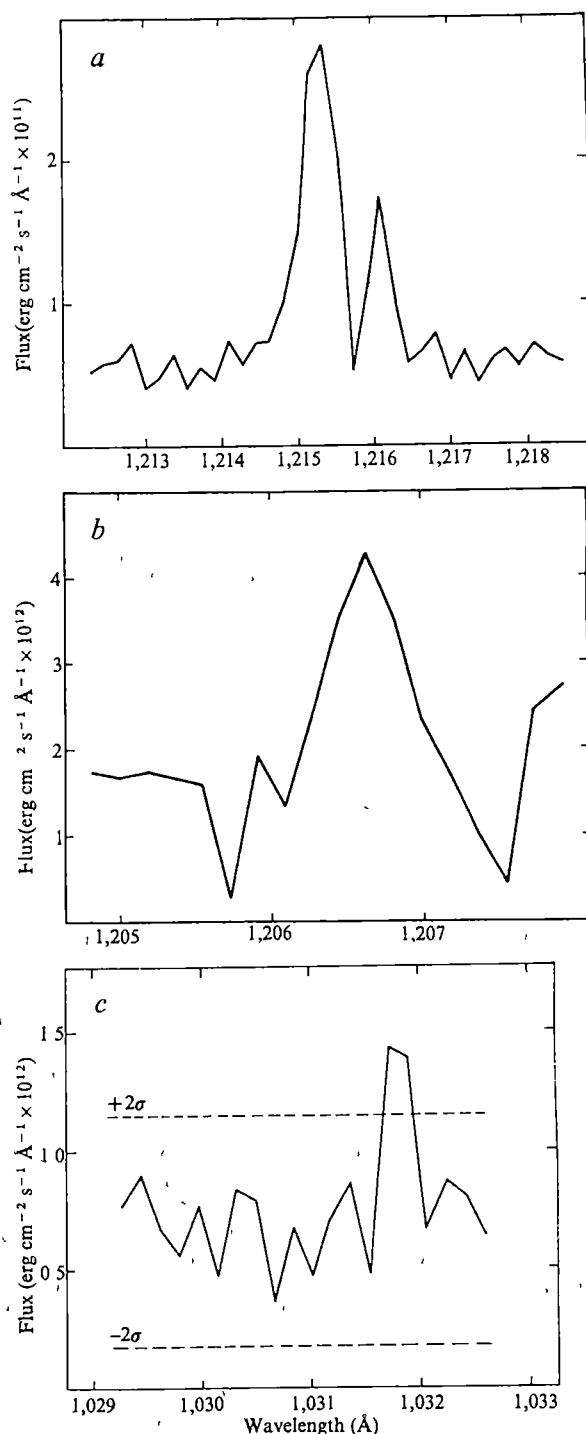


Fig. 1 The observed emission lines of a, H Lyman- α at 1,216 Å, b, Si III at 1,206 Å, c, O VI at 1,032 Å

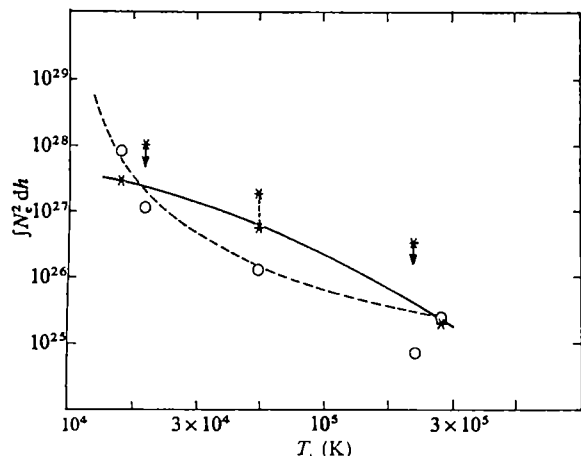


Fig 2 The emission measures, including upper limits, derived from the observed fluxes from Procyon. The corresponding solar values and the mean solar values are also shown. \circ , Sun, \star , Procyon

ground state population is uncertain because of the existence of a metastable level. Interpolating between the observed values gives an emission measure distribution as a function of temperature. This can be used to derive the range of models which would satisfy the emission measures¹⁰ and also fit on to the model of the low chromosphere of Procyon made by Ayres *et al.*¹¹ These models depend on the boundary value of the electron pressure at $T_e = 3 \times 10^5$ K (where O VI is formed) which cannot be determined directly from the present observations. But an upper limit of $N_e T_e = 1.7 \times 10^{14} \text{ cm}^{-3} \text{ K}$ is established by assuming the same total pressure as derived by Ayres *et al.*¹¹, at the lower temperature of 8×10^3 K and a lower limit of $N_e T_e = 2.0 \times 10^{13} \text{ cm}^{-3} \text{ K}$, is obtained by assuming that O VI is formed in an isothermal atmosphere at $T_e = 3 \times 10^5$ K. The chromosphere—or transition region—of Procyon is therefore less dense than the corresponding parts of the solar atmosphere but must be thicker in order to match the emission measures.

The models derived for the two limiting cases have been examined for energy balance. The high pressure model shows the existence of a true transition zone, a region where conductive energy flow is dominant, indicating the presence of a corona at greater heights. But the onset of the transition region occurs at a significantly higher temperature (10^5 K) than in the case of the Sun (3×10^4 K). The low pressure model shows no such transition zone, the conductive flux being small throughout the observed temperature range. Radiative losses must therefore be balanced by the direct dissipation of the primary mechanical energy flux, probably shock heating by acoustic waves, and in this sense the model is better regarded as an extended chromosphere.

So the chromosphere of Procyon extends to higher temperatures than in the Sun, but the detection of a true corona will need additional investigations. A lower limit to the maximum temperature of any corona is given by the temperature of the highest excited line observed (O VI) $T_{\text{max}} \lesssim 3 \times 10^5$ K. An upper limit cannot be derived directly from the observations but overall energy considerations give $T_{\text{max}} \lesssim 6 \times 10^6$ K.

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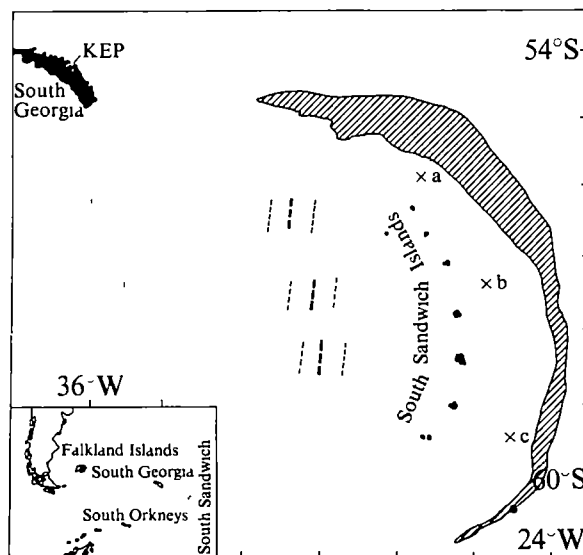
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Seismic wave attenuation and velocity anomalies in the eastern Scotia Sea

As part of a more general investigation of the structure and plate tectonics of the Scotia Sea early in 1972 a temporary seismographic station was installed at King Edward Point, the British Antarctic Survey station on South Georgia (see Fig 1). The station (KEP) comprises a three-component set of short-period Willmore seismometers at position $54^\circ 28' \text{ S}$, $36^\circ 48' \text{ W}$, and two single channel (vertical) outstations with telemeter links to KEP, all five channels record on magnetic tape. Interest has been concentrated on a study of activity associated with the South Sandwich Islands subduction zone¹.

Figure 2 shows typical vertical component seismograms observed at KEP for three earthquakes in the South Sandwich Islands. Event (a) is located in the north, event (b) in the centre and event (c) towards the southern end of the arc (see Fig 1). On replay a bandpass filter of 0.5–10.0 Hz was used for all

Fig 1 The East Scotia Sea. The hatched area represents the South Sandwich Trench at depths greater than 6,000 m. The spreading centre, identified by magnetic anomalies, is shown as thick dashed lines with the 1 Myr isochron shown as a thin dashed line. From bathymetric data the spreading centre is assumed to extend to the trench in the north, and southwards to the South Scotia Ridge. a, b and c, Locations of the three earthquakes discussed in the text.



records. Event (a) is at a range of 5.54° and the seismogram shows distinct P and S phases. On the seismogram of event (b), at a range of 6.78° , there is no clear onset of the S-wave phase but some emergent, low frequency energy can be seen around the expected S-wave arrival time. There is no sign of any S-wave energy on the record of event (c) at a range of 8.09° . These features are also observed on both of the horizontal component records of each event.

Figure 3 shows the P-wave arrivals from each of the three events in detail. The attenuation of the higher frequency P-wave energy from the central and southern events is quite striking; the dominant frequency is reduced from approximately 5 Hz for event (a) to approximately 1 Hz for event (c).

For relocation purposes the arc was divided into three regions: $55-57^\circ$ S, $57-59^\circ$ S and $59-61^\circ$ S. For each of these regions, approximately 20 large, well recorded events (post 1964) were selected and relocated using a Joint Epicentre Determination program². This reduces source bias and determines a set of station corrections for each of the three regions³. Subsequently, events were relocated using a single event program, with the appropriate station corrections applied. This procedure has been applied to all events which occurred after 1964 and it

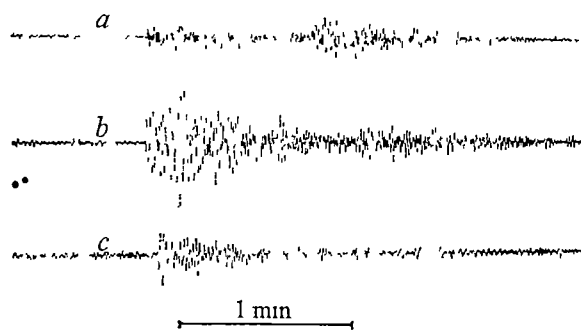


Fig 2 Typical vertical component wave forms observed at South Georgia for the three events shown in Fig 1. Record a is at quarter gain.

results in a marked improvement in clustering of the hypocentres. Since we are here concerned with local travel-time anomalies we have not used the South Georgia data in the relocation procedure. Phase data have been taken from the United States Coast and Geodetic Survey Earthquake Data Report lists, and also from additional station bulletins.

The travel-time residuals at KEP, relative to Jeffreys-Bullen times, of 36 recent, relocated events have been plotted against epicentral latitude (Fig 4). The northern group of 25 events show negative residuals, with a mean value of -4.0 ± 1.4 s. The nine southern events, on the other hand, have large positive residuals with a mean value of $+6.3 \pm 1.1$ s. The residual scatter within the groups is probably a result of the fact that only distant stations have been used for location (South Pole, SPA, at an average range of 3,700 km is often the nearest station), giving poor discrimination between depth and origin time. So far, the number of events recorded from the central region of the arc has not been sufficient to allow accurate mapping of the transition from negative to positive residuals,

Fig 3 Detail of P-wave arrival for the events shown in Fig 2. Record a at quarter gain.

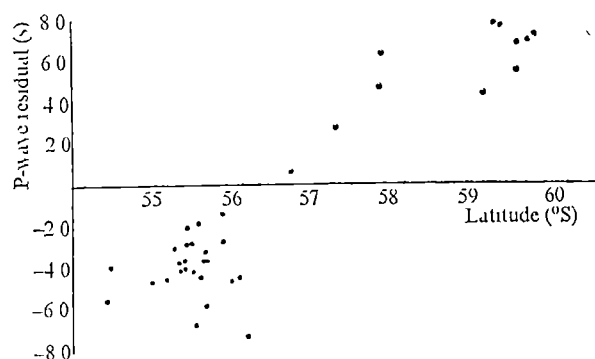
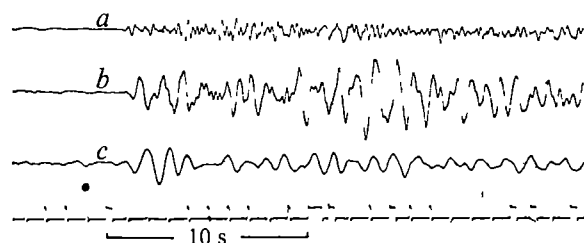


Fig 4 P-wave travel time residuals observed at KEP plotted against epicentral latitude.

however, the few available results suggest that the transition is abrupt. The variation in the residuals over the length of the arc is considerable and indicates a mean velocity difference of approximately 10% between the northern and southern ray paths.

Karig¹ has suggested that the basins behind the western Pacific arcs are of extensional origin, and have been formed by diffuse upwelling of hot mantle material. This has been supported by the discovery of a low Q , low-velocity zone behind the Tonga Island Arc^{5,6} where an upper mantle velocity of 7.1 km s^{-1} has been observed beneath the Lau Basin. This compares with an upper mantle velocity of 8.45 km s^{-1} beneath the old Pacific Basin to the east of the Tonga Arc⁶. In the region under study here, Barker⁷ has identified an active spreading centre, orientated approximately north-south along longitude 30° W (see Fig 1), with a spreading rate of $70-90 \text{ mm yr}^{-1}$. For three-quarters of its path the energy from southern events crosses an oceanic area which is less than 8 Myr old, whereas the ray paths from the northern end of the arc pass through old normal, oceanic lithosphere. The anomalous velocities and attenuation observed here are, therefore, very similar to those which occur in the marginal basins of the western Pacific and are consistent with the presence of a high temperature zone of mantle at shallow depth. The similarity is interesting in view of the fact that behind the South Sandwich Arc the basin shows all the usual features of mid-oceanic ridge spreading.

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Manganese ore deposits and plate tectonics

It is now generally accepted that the oceanic crust and overlying pelagic sediments are enriched in several metals, including copper and manganese¹⁻³. The theory of plate tectonics has demonstrated that the oceanic lithosphere is likely to be remobilised or emplaced along convergent continental margin through magmatic or tectonic activity. The plate tectonics model has been used to explain the metallogenesis of copper⁴⁻⁸ and these concepts have been extended to manganese mineralisation along orogenic belts^{9,10}. We demonstrate that plate

consumption along subduction zones gives rise to at least three processes which may be associated with production of manganese deposits

One type of manganese mineralisation is related to calc-alkaline magmatism. Reports on porphyry copper deposits and numerous manganese ore deposits have revealed that the two mineralisations seem to be contemporaneous within calc-alkaline terrains¹¹. Porphyry copper deposits consistently occur over active and recently active subduction zones throughout the circum-Pacific, Caribbean, and Alpine orogenic belts, except in Japan where stratabound copper sulphide deposits predominate¹². Manganese mineralisation is also found in these Mesozoic and Cainozoic porphyry copper deposits (including Japan), as well as older terrains (Palaeozoic and perhaps Precambrian) from which porphyry copper deposits have presumably been eroded. Porphyry copper deposits are usually found in calc-alkaline stocks and cupolas underlying stratovolcanoes, although copper mineralisation is a rather late event in the emplacement of the entire calc-alkaline suite⁴⁻⁸. Similarly, many manganese deposits are also closely associated with calc-alkaline igneous rocks, particularly andesitic tuffs and breccias, usually in the vicinity of volcanic centres. Furthermore, these manganese deposits seem to be formed after the main phase of volcanic activity¹³. Sillitoe⁷ has proposed a plate tectonic model for the origin of porphyry copper deposits, which embodies the concepts of seafloor spreading, transform faulting, underthrusting at continental margins and island arcs, and the generation of calc-alkaline magmas by partial melting of oceanic lithosphere. He has also suggested⁸ that typical porphyry copper deposits occur in the upper levels (at depths of 1.5–3 km beneath the summits of stratovolcanoes) of porphyry copper systems which may possess vertical extensions as great as 8 km.

The close space-time relationship between manganese deposits, porphyry copper deposits, and subduction zones indicate that both manganese and copper are incorporated into melts which rise to form calc-alkaline volcanic chains and co-magmatic roots⁹⁻¹¹. These magmas are generated on or above Benioff zones by partial fusion of oceanic crust rich in metals and pelagic sediments, and incorporate volatiles derived from subducted oceanic lithospheric plates¹⁴⁻¹⁸. After the metals become incorporated into the magmas forming the roots of volcanic chains, solutions from which ores may form are expelled from cupolas of these roots, leading to brecciation and intense hydrothermal activity characteristic of porphyry copper systems. The metals are probably transported in hydrothermal fluids and solutions as complex Mn(II) and Cu(II) ions. At lower temperatures in aqueous environments, hydrolysis occurs leading to the precipitation of extremely insoluble sulphide, oxide and metallic phases of Cu. Manganese is transported to higher levels in the porphyry copper system and may escape out of the volcanic pile into the submarine environment where it is deposited as Mn(II) carbonates and silicates, or where oxidation to very insoluble Mn(IV) oxide minerals takes place.

A second location of manganese ore deposits is in ophiolites, which comprise, in ascending order starting from the stratigraphic bottom, ultramafic bodies, complex gabbro intrusives, basalt pillow flows and sediments. This sediment layer, although metamorphosed to varying degrees, frequently contains substantial manganese deposits. Examples of such mineralisation are found throughout the western coast of North America and throughout the western circum-Pacific, particularly in Japan. The Alpine belt is also well endowed with manganese deposits in ophiolites, particularly in Morocco. Other examples include Cuba, the Urals, and eastern Australia. The ages range from the Precambrian to the Cainozoic. The remarkable resemblance of ophiolites to oceanic floor, both in terms of chemistry and geometry, has prompted the widespread belief that ophiolites are indeed oceanic floor, emplaced tectonically into orogenic belts^{19,20}. Ophiolite emplacement is integrally associated with plate subduction, either by the accretion of

oceanic crust on to the wall of the upper plate (in oceanic trenches) or by the bodily thrusting (obduction) of the oceanic crust on to a continental margin¹⁹⁻²². The principal difference between the two mechanisms is the direction of subduction relative to the continental margin. Both cases are associated with blueschist (glaucophanite) metamorphism and melange terrains.

The widespread occurrence of manganese in the uppermost layers of ophiolites is understandable when one considers the abundance of manganese in the sedimentary layers of the oceanic crust. Deep sea drilling projects^{23,24} and geochemical studies across oceanic rises²⁵⁻²⁷ have revealed a sediment layer rich in manganese oxide apparently generated by the volcanism occurring at the rises. Oceanographic cruises have mapped extensive areas of ferromanganese nodule deposition on the sea floor, particularly in the north-east Pacific Equatorial Region²⁸⁻³⁰. These sediments, or their metamorphosed equivalents, are frequently, if not typically, associated with ophiolite terrains.

A third location of extensive manganese mineralisation exists within the small marine basins located behind island arcs. Examples include the Philippine Sea and the Sea of Japan. One occurrence of a manganese deposit in an existing marginal basin is the Mariana Trough near Guam in the western Pacific³¹. This was a 6 cm wide vein of manganese oxide containing 48.0 weight % Mn. But a larger scale example is the Atasu region in central Kazakhstan, USSR, which we interpret as an uplifted Palaeozoic marginal basin.

Marginal basins are thought to be produced by thermal diapirs rising off subduction zones at depths of 200 to 400 km (ref. 32). These thermal diapirs are essentially rising zones of high heat flow generated at the surfaces of subducted lithospheric slabs. As a diapir rises upward, it upwells and spreads outward so as to force the basin floor apart. The high heat flow near the surface of the basin triggers volcanism which fills the widening rift with basaltic magmas, thereby producing new oceanic crust. Marginal basins are therefore bounded by steep scarps marking the original walls of the initial rift valley^{21,33}. As spreading commences and sediments rapidly fill the developing trough, continued normal faulting pervades both the new basaltic crust and the overlying sediments. Except for the volcanogenic andesitic flysch apron at the arc side, the marginal basin represents the typical miogeosynclinal environment of manganese deposition. By analogy with the processes occurring at oceanic rises, the median rifts of marginal basins are expected to be the sites of manganese emanations, although the median rifts will be overlain by a thick and highly faulted sequence of sedimentary materials. Solutions rich in manganese reach the surface through the fault conduits. Indeed, the manganese mineralisation in central Kazakhstan is intimately related to the normal faulting which characterises the complex Kazakhstan graben³⁴. Continuous sedimentation accompanied the spreading of the central Kazakhstan trough so that the oldest sediments are now found buried at the flanks of the Palaeozoic basin. As seafloor spreading continued, sedimentation progressively buried newly formed crust and became the host of manganese mineralisation.

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Magnetic susceptibility stratigraphy of Pacific Pleistocene sediments

FROM studies on a few gravity cores¹ it is known that the absolute susceptibility of Pacific sediments ranges from 10^{-5} to 10^{-6} e m u g⁻¹ oersted⁻¹. Using a simple, non-destructive and rapid logging technique², we measured the relative susceptibility, as a function of depth, in 86 gravity cores from the Pacific (Core Library, Scripps Institution of Oceanography). We report the salient features of this investigation (details will be presented elsewhere).

Figure 1 shows the location of the cores and Fig 2 the three types of susceptibility logs obtained. Type 1 occurs mostly in the trench and sediment trap areas, each peak in the magnetic susceptibility (Fig 2) probably representing an episode involving the deposition or slump rich in volcanic material. Such events, which occurred in

Fig 1 Location of cores in the Pacific. Dashed double lines, principal trench systems. ●, Type 1 logs, ×, type 2, ■, type 3.

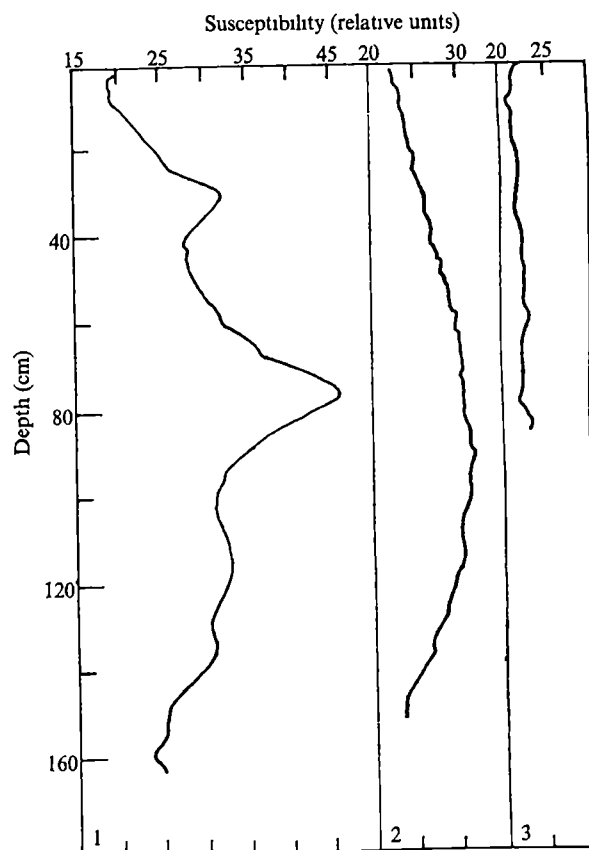
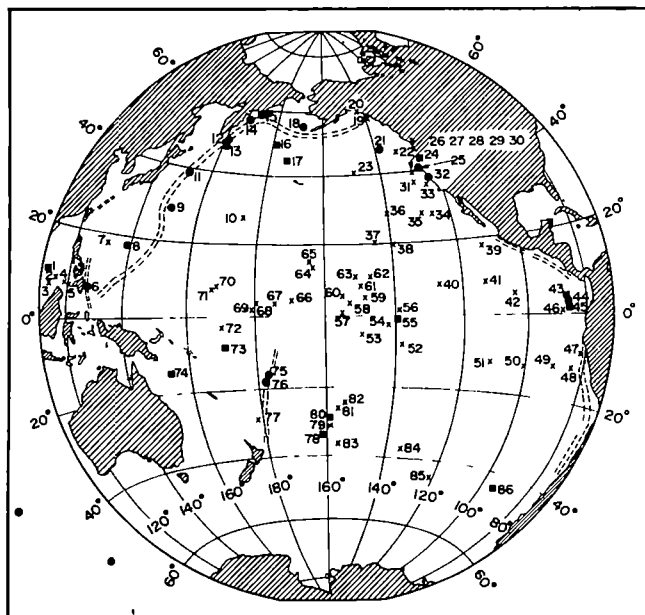


Fig 2 Typical examples of the three types of log. Number underneath each curve indicates the type.

the period 0.2–2.8 Myr ago, were sometimes synchronous in large areas representative of type 1 and sometimes localised.

Type 2 are the most common, 66% of the cores studied belong to this type and have a single, broad hump in the susceptibility log (Fig 2), the maximum of which occurs anywhere between 40 cm and 120 cm. These events occurred in the period 0.2–2.5 Myr ago.

Four explanations are possible, one of which is the oceanwide deposition of volcanic material. Regions containing sediments of type 1, by virtue of their proximity to the volcanic activity, register a sharp event and the sediments from the rest of the Pacific basin register only a diluted or smeared event which may appear as a broad hump covering the same time span. An increase in the amount of atmospherically transported magnetic material (cosmic, volcanic and terrestrial)³ deposited on the Earth in the past, could be responsible, an observation supported by studies on slow-growing manganese nodules^{4,5} and meteorites⁶. A decrease in the accumulation rate of sediments, which would in effect manifest itself as an increase in the magnetic material depositing at that time, is another alternative. The hump could also result from slow chemical or physical changes after deposition, which may affect, for example, the composition or state of seawater itself over the time period represented by the hump. To ascertain the relative importance of these explanations, detailed work involving mineralogy and geochronology of the cores of type 2 is necessary.

About 15% of the cores belong to type 3, in which the susceptibility is approximately constant with depth (Fig 2). A few of these cores also show a single, broad hump, as for type 2 but the hump is very flat and is distinguishable from the former.

The accumulation rates of the Pacific sediments had to be ascertained from the literature as none of these cores was dated. Some of the cores, especially of types 1 and 2 could be dated using beryllium-10 (ref 7) and ionium/thorium techniques⁸. The maximum of the broad hump in type 2 seems to be a fairly well distributed event in the whole Pacific and may act as a stratigraphic marker. To detect this marker in a gravity core (about 150 cm) takes no more than half an hour. As has already been stressed, such a log will also indicate the suitability of a core for palaeomagnetic reversal studies², types 2 and 3 are ideal. Further investigations of this kind on piston and Deep Sea Drilling Project cores, together with the mineralogy and geochemistry, may prove very useful for establishing the magnetic susceptibility stratigraphy of the world ocean sediments.

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Late Pleistocene tropical aridity synchronous in both hemispheres?

THERE IS now firm evidence of late Pleistocene aridity in many parts of the tropics in contrast to the belief that high latitude glacial periods were coeval with low latitude pluvial periods¹. A good case may be made for synchronous glacial conditions in both hemispheres during the late Pleistocene^{2,3}, although there are notable and intriguing exceptions⁴. The latter may result from precipitation variations caused by changes in the incidence of local rain-bearing winds, which in turn result from changes in sea level, ocean currents, and, perhaps, from rain-shadow effects. It is reasonable to hypothesise that major climatic changes in extraglacial areas, in particular the non-mountainous parts of the tropics, may also have been synchronous during the late Pleistocene. We propose that the increasing evidence from Africa, India, South America and Australia provides further support for the view that there was widespread tropical aridity in both hemispheres during the late Pleistocene. We will review evidence from the Northern Hemisphere first.

In East Africa, lake levels rose at the start of the Holocene after a long period of low levels and high salinities^{5,6}. Lakes Victoria and Albert in Uganda were low for an unknown time just before 12,500 yr BP (refs 7 and 8), and the late glacial vegetation on Ruwenzori⁹ was significantly less mesophytic

(adapted to a moderately moist environment) than during the Holocene. Lakes in the Kenya Rift Valley, high during the Holocene, were low immediately beforehand^{10,11}. Lake Rudolf on the Kenya-Ethiopia border, fed by the Ethiopian Omo river, was low before 12,000 yr BP and high thereafter⁶. In the southern Afar Rift the lakes were low and saline between about 20,000 and 12,000-14,000 yr BP, rising during the early Holocene^{12,13}. Lake Chad, fed by the Logone and Ubangui-Shari river systems, was very low between 20,000 and 13,000 yr BP, and high from 12,000 to about 5,000 yr BP (ref 14). Rainfall on to and runoff from the Adamawa Mountains in Cameroon must therefore have been low during the late Pleistocene.

More direct evidence of late glacial tropical African aridity comes from the extensive belt of fixed dunes which now extend down to latitude 10°-12° N in Senegal, Mali, Niger, Nigeria, Chad and Sudan¹⁵⁻¹⁹ over an east-west distance of some 5,000 km. Until the recent drought, self dunes were mainly active in this region north of the 150-mm isohyet¹⁷. The fixed dunes extend south to about the 800-mm isohyet¹⁷, and calculated palaeo-wind directions suggest a late Pleistocene southward shift of the sand-moving winds in Sudan of about 200-450 km¹⁹. At Adrar Bous in the Tenere desert of Niger, wind-patinated Aterian tanged points and associated stone tools, firmly dated elsewhere at between about 30,000 and 15,000 yr BP, coincide with a phase of aridity which was followed and preceded by periods of high lake levels in this now dry area^{20,21}. In the valley of the White Nile in central Sudan, linear dunes were active shortly before 12,000 yr BP, when they became partly submerged beneath fluvio-lacustrine clays deposited by the rising White Nile^{22,23}.

There is also abundant evidence of widespread (?) late Quaternary aeolian activity in parts of Angola, Zambia, Mozambique, Rhodesia and Zaire²⁴⁻²⁶, although the exact age of these wind-blown sands is still in doubt. The pollen spectra from Angola²⁷ and from Kalambo Falls²⁸ in Zambia, have been better dated, but changes in the ratio of tree to non-tree pollen are related to both rainfall and temperature, and an increase in grassland at the expense of woodland may reflect lower glacial temperatures rather than reduced precipitation. Equally, although the geomorphic evidence for late Pleistocene aridity in west-central Zaire²⁵ seems valid, we do not yet know whether this aridity is related to slight changes in the cold Benguela Current²⁹, or is in fact more general. A recent reappraisal of published pollen spectra from tropical Africa³⁰ tried to separate inferred temperature changes from inferred changes in humidity. The writers concluded that during the late Pleistocene dry conditions prevailed at Sacred Lake (Mount Kenya), the Cherangani Hills (Kenya), Muchoya swamp (on Ruwenzori) and in the Lake Victoria basin (Uganda).

In the southern Rajasthan desert of north-west India, salt lakes were full of freshwater, and the vegetation was locally mesophytic from at least 10,000 to about 4,000 yr BP (refs 31 and 32). Some of the lakes were formed by the late Pleistocene damming of former river courses by aeolian sands, and wind-blown sands extended as far east as the 850-mm isohyet during the late Pleistocene and at least once beforehand³³⁻³⁵. Late Pleistocene aridity in tropical north-west India therefore seems plausible.

Data from Australia are sparse but informative. In north-west Australia stable self dunes now extend out on to the continental shelf. Holocene shallow-water marine and estuarine clays unconformably overlie these dune sands, which lack a soil profile and so were probably active until the Holocene³⁶. In deep-sea core V-260 on the Sahul Shelf between Timor and Darwin the rate of clastic sedimentation was rapid during the late Pleistocene and slow thereafter. The carbonate nodules which occur in the Pleistocene but not in the Holocene portions of the core are regarded as pedogenic and as symptomatic of a soil climate drier than the present one^{37,38}. At Lynch's Crater in north-east Queensland a pollen core extending over 60,000 yr indicates a change from a less mesophytic to a more mesophytic regional vegetation from the late Pleistocene to the early Holocene³⁹, and is the most convincing evidence for late

Pleistocene aridity in tropical Australia. Theoretical reconstructions of the late Pleistocene climates of the Torres Strait area in northern Queensland also suggest late glacial aridity⁴⁰.

Deep-sea cores from the continental shelf east of Brazil show rapid deposition during the very late Pleistocene of abundant unweathered feldspar and coarse sub-angular quartz sand, changing abruptly to fine grained marine deposition during the Holocene⁴¹. Both Holocene marine cores⁴¹ and modern sediments carried by the Amazon⁴² contain relatively little feldspar, suggesting that chemical weathering was limited and mechanical erosion dominant during the late Pleistocene on the Precambrian Shield areas of Brazil and Guiana, an inference consistent with a climate drier than the present. Pollen studies in lowland tropical South America⁴³ likewise reveal a change from late Pleistocene savannah woodland to early Holocene rain forest, much as in Queensland³⁹ and Uganda⁹.

The final line of evidence concerns aeolian detritus in deep-sea cores from the tropical Atlantic Darwin (in 1946) and Ehrenberg (in 1847) correctly interpreted wind-blown dust in the tropical mid-Atlantic as being of African and probably of Saharan provenance⁴⁴, a conclusion confirmed recently⁴⁴⁻⁴⁹. The importance of aeolian deposition of continental dust in islands as far afield as Barbados⁴⁶ and Hawaii⁵⁰ is well documented, as is the significant aeolian contribution to deep-sea sedimentation⁴⁴⁻⁴⁷. Comparisons between well dated tropical cores for which ¹⁸O/¹⁶O palaeotemperature curves exist show that temperature minima coincide with high concentrations of freshwater diatoms and opal phytoliths⁵¹, as well as a large increase in the proportion of relatively unweathered clastic mineral particles^{41, 52, 53}. The published data show high rates of deflation and mechanical erosion during the late Pleistocene in both tropical Africa⁵¹ and Brazil⁴¹.

Taken together, the evidence from pollen analysis, geochemistry, geomorphology, marine geology, and isotope chemistry is entirely consistent with low latitude aridity in Africa, India, South America and Australia during the uppermost Pleistocene. The essential problem now is to select, from among the many models^{24, 29, 40, 41, 54}, a mechanism capable of reducing effective precipitation throughout the tropics during the latter stages of an ice age.

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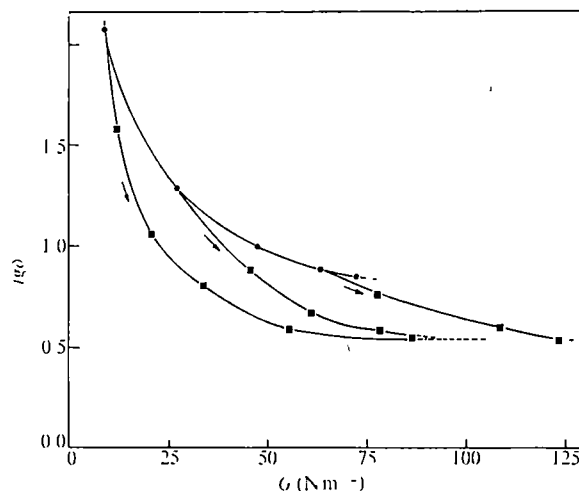
Structural hysteresis

SINCE hysteresis was first encountered in magnetism, various examples have been reported in different branches of science, as in mechanics and in surface chemistry. A unified mathematical approach to all these phenomena is possible by means of the theory of system dynamics. Such a theory shows that two time-dependent characteristics, not related by a one-to-one correspondence, will be shifted with respect to each other when they are subjected to periodic change. This phase lag causes hysteresis.

In mechanics, thixotropy is another typical example. It describes materials whose resistance to flow is lowered when subjected to mechanical agitation. In such a system the shear stress, τ , will normally not be in phase with a shear rate, $\dot{\gamma}$, that is varied periodically. Hence a τ - $\dot{\gamma}$ plot will show a hysteresis loop¹⁻³. A related, but more general, type of hysteresis in mechanics has been mentioned by Frederickson⁴.

Although thixotropy has been known⁵ for more than 50 years, no satisfactory analysis is available. Some physical measurements^{6, 7} substantiate a qualitative explanation based on reversible, shear-induced changes of structure. Its application in a quantitative description has been limited to a few special cases^{8, 9}. Cheng has presented a general framework for the phenomenological analysis of inelastic thixotropy in which

Fig 1 Mechanical loss tangent as a function of shear modulus from measurements made at 0.5 Hz. ●, Equilibrium under steady state shear flow, ■, recovery under rest after shearing



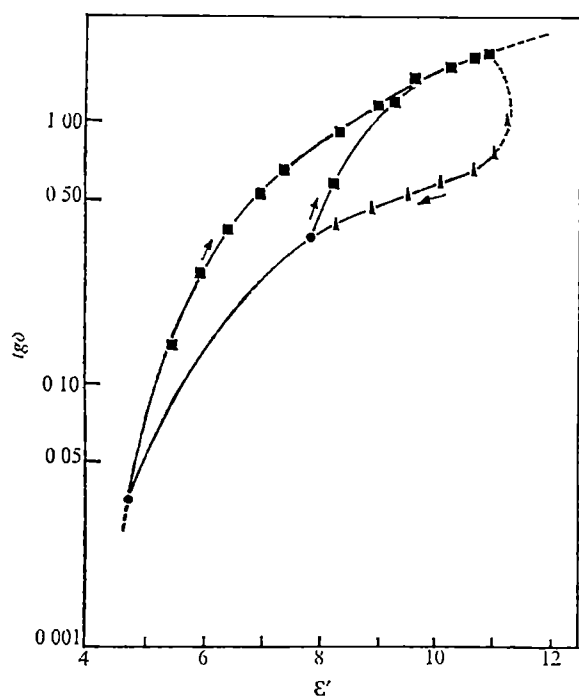


Fig 2 Dielectric loss tangent as a function of dielectric constant from measurements made at 1.6 kHz: ●, Equilibrium under steady state shear flow, ■, recovery under rest after shearing, ▲, breakdown under shearing

most of earlier attempts can be fitted¹⁰. Two equations are required. One is a constitutive equation relating the instantaneous value of viscosity, η , to the shear rate and to the instantaneous degree of structure through a parameter, λ , $\eta = f(\dot{\gamma}, \lambda)$. The other is a kinetic equation relating the rate of change of the instantaneous degree of structure, $d\lambda/dt$, to the shear rate and the instantaneous degree of structure $d\lambda/dt = g(\dot{\gamma}, \lambda)$.

An important feature of the theory is the assumption that structure can be characterised by a single parameter. Although Cheng has pointed out that this is not essential to his theory, it seriously affects the application of the theory. The general applicability of a single parameter description is of fundamental and technological importance. Yet, as far as we are aware, it has never been considered in detail. Ruckenstein and Mewis⁹ have argued against it on theoretical grounds, other investigators have done the same, using arguments based on experimental evidence^{11,12}.

Clarification is possible if several properties of the changing structure are measured simultaneously. For the one-parameter assumption to be valid, there should be a discrete one-to-one correspondence between all of them.

A mechanical investigation of thixotropic systems in the oscillatory, low frequency mode often shows a viscoelastic response^{13,14}. In that case the real and imaginary components of the modulus provide two distinct characteristics of the structure. For a chrysotile dispersion a unique relationship between both has been found in the test conditions used¹⁴. A thixotropic polyamide gel, however, revealed a deviating behaviour¹³.

We now report similar experiments using a dispersion of 3.6% carbon black in mineral oil. The dielectric properties of such a system are known to depend on structure¹⁵. Measurements in the dielectric dispersion region provide a dielectric constant with a real, ϵ' , and an imaginary, ϵ'' , part. In Figs 1 and 2 we show mechanical and dielectric data for breakdown and recovery of structure.

In both figures different paths are followed during recovery and breakdown so a single parameter theory seems inadequate for the description of thixotropy in this material. The results indicate that not only the degree of structure but also the kind of

structure depends on the shear history. We suggest that the consequent hysteresis be called 'structural hysteresis'. A quantitative discussion requires a systematic investigation of the changes in viscoelastic and dielectric spectra, which is under way and will be reported elsewhere.

But the present data indicate that not all interparticle bonds have similar effect. As a first approximation the behaviour could be understood by assuming spherical agglomerates to be linked by particle chains in a three dimensional network. When shear is applied the interparticle bonds in the chains are more susceptible to rupture and so break down first. Subsequently, depending on the shear rate, the size of the spherical agglomerates decrease. When the shear is discontinued the more probable chain links develop first and give rise to a loose network, which slowly repairs its original structure. By changing the shear history distinct paths can be followed in plots like those in Figs 1 and 2 to go from one structure to another. We believe this is of more general kinetic and thermodynamic interest in colloid science. This picture also accounts, at least qualitatively, for some of the discrepancies in the kinetics of structural changes in thixotropic materials^{11,12}.

These results might also contribute to a better understanding of the nonlinear behaviour of other systems, such as polymers, where simplified thixotropic theories have already been applied^{16,17}.

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Dispersion of particles by shear

It is generally accepted that in the hydrodynamic dispersion of particles in liquids, shearing motions—including those produced in turbulent flows in which the viscous forces are dominant—play a central role. We draw attention here to the failure in existing theoretical treatments¹ to recognise that there are profound differences in the efficacy of different kinds of shear, and specifically between simple (or transverse) and pure (or extensional) shears.

This is illustrated by considering the two shearing flows

$$U_1 = Gx_2 \quad U_2 = 0 \quad U_3 = 0 \quad (1)$$

$$U_1' = Gx_1'/2 \quad U_2' = -Gx_2'/2 \quad U_3' = 0 \quad (2)$$

where U_1 is particle-free liquid velocity parallel to the X_1 -axis

The transverse shear of gradient G given by equation (1) has a rotational rate $G/2$ about the vorticity axis X_3 whereas the two dimensional flow described by equation (2), one case of extensional flow, is irrotational, the two fields, however, are identical when $X_3' (\equiv X_3)$ is rotated at $G/2$ at the instant when X_2' lags X_2 by 45° (refs 2, 3)

The simplest case of dispersion is that of a pair of rigid neutral spheres, without mutual attraction and repulsion, and with negligible Brownian motion. In field (1) they revolve in closed trajectories around one another, so that they are not mutually dispersed, as has been observed experimentally and explained theoretically⁴⁻⁷. By contrast, in field (2) (generated experimentally in a four-roller apparatus^{2,3}) we have observed with 1 mm polymethylmethacrylate (PMMA) spheres in 100 poise silicone oil that a pair of spheres always separates as predicted (P. A. Arp and S. G. M., unpublished) except when the spheres are in physical contact. This important difference results from periodic rotation of the pair in field (1) but not in field (2), for the latter flow the line joining sphere centres asymptotically approaches the X_1' direction and the separation

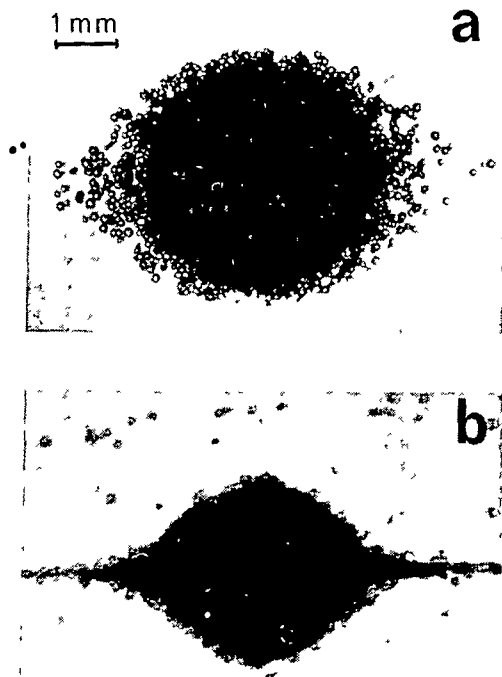


Fig 1 Photographs showing aggregates in *a*, simple shear flow, *b*, extensional flow at $G = 0.44 \text{ s}^{-1}$ and $t = 7 \text{ min}$. In both photographs the X_1, X_1' axes are horizontal, and the X_2, X_2' axes are vertical

steadily increases. A similar difference in behaviour from the rotational field (1) may be expected in three dimensional (non-rotational) extension

$$U_1' = -Gx_1'/4 \quad U_2' = -Gx_2'/4 \quad U_3' = Gx_3'/2 \quad (3)$$

Another striking difference is demonstrated by a long elastomer filament. In field (1) the filament gradually coils up⁸ but the opposite happens in field (2), that is, a coil becomes extended into a straight thread aligned along the X_1' axis

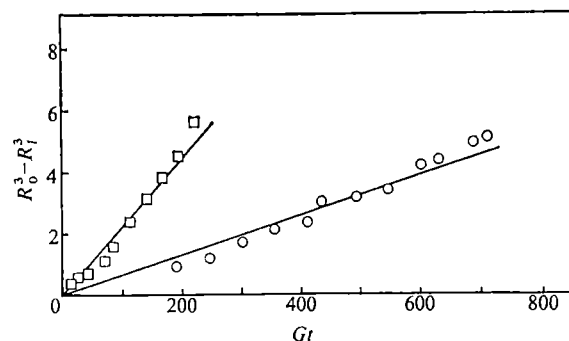


Fig 2 Experimental test of equation (4) for aggregates like those in Fig 1 for shear flow (O, $k = 7 \times 10^{-6} \text{ cm}^3$) and extensional flow (□, $k = 22 \times 10^{-6} \text{ cm}^3$)

In simulating the dispersing process, we have carried out experiments with non-cohering aggregates consisting of concentrated suspensions, estimated at 60 volume per cent, of 100 μm PMMA spheres in silicone oil rolled into spherical balls some 3 mm in diameter. In field (1), the aggregates rotate integrally with a periodic wobble similar to viscous liquid drops of zero interfacial tension⁹, but with no sign of immediate breakup and dispersion. Instead, individual spheres from the peripheral layer gradually disengage near the regions at 45° and 225° in the direction of rotation from the X_2 axis where the extension rate is greatest. After leaving the aggregate the particles continue to circle around it and seem to be bound by the closed streamlines similar to those around a single liquid drop¹⁰. Not until this closed streamline region becomes crowded with individual particles do they start being dispersed in the surrounding fluid (Fig 1a). At the corresponding G in the four-roller apparatus, on the other hand, the aggregates disintegrate much more rapidly with the spheres being pulled directly off the outer layers from the onset of flow and dispersed in the $\pm X_1'$ directions (Fig 1b).

A simple analysis of the breakup, in which it is assumed that the number rate at which spheres are pulled off the periphery of the aggregate is proportional to the tensile stress generated by the sheared liquid at a given point (relative to the fixed coordinate axes) on the surface of the aggregate, leads to the following approximate relationship between the aggregate radius R_t and the shearing time t

$$(R_0^3 - R_t^3) = kGt \quad (4)$$

Equation (2) holds reasonably well (Fig 2) with the rate constant k , which is a measure of the dispersing efficiency, for field (1) being about 1/3 of that appropriate for field (2). This marked inferiority of field (1) is undoubtedly caused by rotation of the aggregate which causes tensile forces acting on the individual particles to reverse to compression before the particles disengage. In field (2), on the other hand, the tension (existing between $\pm 45^\circ$ of the $\pm X_1'$ axis) remains constant at a given point until disengagement occurs.

We have observed the same general behaviour with flocs of interlocked woodpulp fibres where the breakup is complicated and retarded by the periodic bending of the peripheral fibres in field (1), but which proceeds quickly, with the fibres aligning along X_1' in field (2).

Similar considerations should apply to other processes and phenomena such as (1) fibrous crystallisation of polymers from melts and supersaturated solutions¹¹, (2) breakup of liquid drops² and the emulsification and homogenisation of liquids, (3) mixing and blending of mutually soluble polymer melts and other liquids, (4) probable breakup of erythrocyte rouleaux and other blood cell aggregates in convergent (partially extensional) flows in blood vessels. In the examples

cited, vorticity can have an inhibitory effect. This principle seems to have been realised empirically in rolling mills for rubber, inks, paints, pastry and so on, but not in any theoretical considerations

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Retention of mercury from an industrial source in Swansea Bay sediments

IN a study of mercury in sediments in Swansea Bay, mercury dispersed from an industrial outfall into the bay is at least partially retained in the sediments within a radius of 2 km. There is a very significant correlation between the mercury content and organic carbon content of sediments ranging from sand to mud over the whole bay, but with a higher ratio of mercury to organic carbon in the contaminated sediments.

High mercury levels in marine sediments close to sewage outfalls have been reported from California¹ and Connecticut², with the implication that these outfalls were sources of environmental mercury. There are, however, natural factors which can cause increased mercury levels in one area relative to another, and in neither study were these factors sufficiently discussed for positive conclusions to be drawn.

Thomas³ demonstrated that for freshwater sediments (Lake Ontario) there is a correlation between mercury content, organic carbon and fine grain size. We have examined the corresponding relationship for marine sediments, and deviations from the relationship which occur close to an industrial outfall.

Swansea Bay, on the South Wales coast, has very varied sediment types, ranging from clean sand with an organic carbon content less than 0.1%, to mud (90% passing through a 63 μ m sieve) with 2% or more organic carbon. An outfall dispersing effluent from a chemicals complex, which includes a chlor-alkali plant, is known to release a small amount of mercury to the bay. The patchy nature of the sediment means that mercury levels vary widely from place to place, and thus isopleths of mercury contents will reflect not the influence of external sources of mercury, but

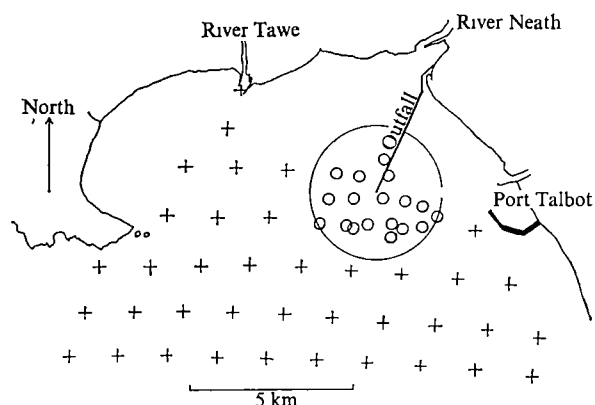


Fig 1 Map of Swansea Bay showing sampling positions. Samples taken within a 2 km radius of the outfall are indicated as circles

sediment type, which is determined by currents, wave action and sediment source.

Fifty-five bottom samples were taken from the bay by Shipek grab 18 from within a 2.0-km radius of the end of the outfall, and 37 from an approximately 1.5-km grid over the rest of the bay. There was a rather higher sampling density around the outfall to enable a statistical comparison to be made between the two sets of samples. The distance of 2.0 km was chosen arbitrarily before sampling. Figure 1 shows the distribution of sampling positions. Samples were freeze dried and disaggregated before analysis, and showed no loss of mercury on freeze drying. Mercury analysis comprised digestion with aqua regia, potassium permanganate and potassium persulphate⁴, followed by flameless atomic fluorescence spectrophotometry, with a Shandon Southern mercury fluorescence unit⁵ modified to fit an Evans Electro Selenium EEL 240 atomic absorption spectrophotometer. Organic carbon was analysed by a chromic acid method, slightly modified⁶ from that of Walkley and Black⁷. Grain size analysis was done by sieving.

For the three parameters, mercury content, organic carbon, and mud fraction, the correlation coefficients were computed (Table 1). In all cases the correlations are high, and, presumably because mercury is directly associated with organic material and thus only indirectly with sediment type, the correlation between mercury and organic carbon is higher than that between mercury and mud fraction. Accordingly, the correlation between organic carbon and mercury was investigated more fully. A plot of organic carbon (percentage dry weight) against the logarithm of the mercury content (in ng per g dry weight) is reproduced in Fig 2. The dotted line represents a fixed ratio of 250 ng mercury per g dry sediment for each percent of organic carbon. All samples from within 2.0 km of the outfall are shown as circles, and the rest as crosses. The dotted line shows that the relationship between mercury and organic carbon content for the Bay samples (crosses) approximates to simple proportionality. The mean ratio, mercury (ng per g) to carbon (%) for all samples is 283, whereas for the two populations of samples the means are 239 ($\sigma=76$) and 381

Table 1 Correlation coefficients

	Mud	All samples Organic carbon	Mercury	Mud	Bay samples only Organic carbon	Mercury	Mud	Outfall samples only Organic carbon	Mercury*
Mud	—	0.91	0.69	—	0.93	0.81	—	0.81	0.50
Organic carbon		—	0.76		—	0.94		—	0.73
Mercury			—			—			—

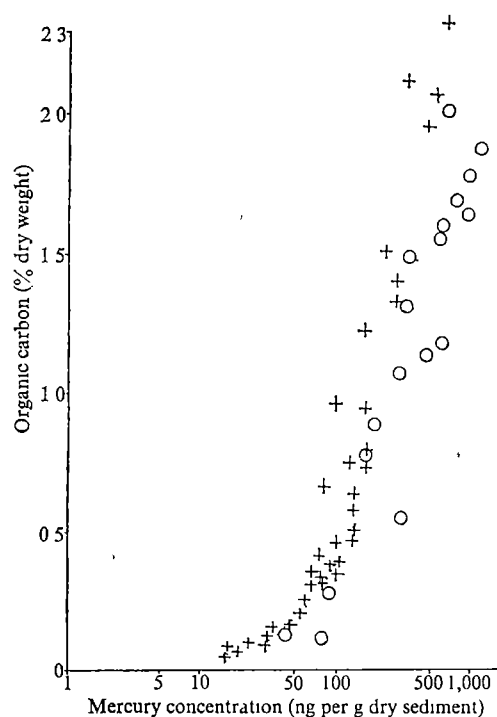


Fig 2 The relationship between mercury and organic carbon content of the sediment samples.

($\sigma=156$) for Bay and outfall respectively. The difference is definitely significant: the difference between the sample means is more than three times the standard error of the difference⁸.

From this we conclude that some at least of the mercury from the outfall is retained in the nearby sediments. There is no other known mercury source in this area of the Bay—the River Neath, which flows in at the north-eastern corner of the Bay, has very low levels of dissolved mercury (typically between 5 and 10 ng l⁻¹). Swansea Bay cannot be called heavily contaminated: the highest level found was 1,600 ng g⁻¹, which is very similar to values reported from Los Angeles¹, New Haven², Nova Scotia³, and Hampshire¹⁰ coastal sediments, some of which were believed to be influenced by sewage discharges. But these values are not as high as, for instance, the 9,000 ng g⁻¹ recorded in Lake St Clair¹¹, which was subjected to known mercury discharges. Thus although no serious pollution is present, the situation should be kept under observation. A detailed statistical analysis of these and other trace metal data for Swansea Bay will be published elsewhere.

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Seasonal rainfall forecasting in West Africa

To provide a basis for the seasonal prediction of rainfall in the Sahel, Winstanley¹ presented the linear regression of June on June–September rainfall for 59 stations. This regression is statistically inappropriate. June rainfall should be taken as the independent variable and not included in both the dependent and independent variables. Though June rainfall accounts for less than 15% of the seasonal rainfall on average, it accounts for a much larger proportion of the variance. The coefficient of

Table 1 Regressions of seasonal rainfall (Y) on monthly rainfall (X), both expressed as % departures from the mean (based on 1931–60 wherever possible)

	Correlation coefficient	Significance level
(a) $Y = -6.0 + 0.129X$	0.22	$P < 0.40$
(b) $Y = -5.2 + 0.251X$	0.44	$P < 0.06$
(c) $Y = -7.2 + 0.291X$	0.64	$P < 0.01$
(d) $Y = -5.8 + 0.327X$	0.41	$P < 0.06$
(e) $Y = -3.3 + 0.239X$	0.26	$P < 0.05$

- (a) 50 stations between 10° and 20° N, 1953–1972, using July to September on June.
- (b) 50 stations between 10° and 20° N, 1953–1972, using June to September on June.
- (c) 59 stations between 10° and 20° N, 1953–1972, using June to September on June (from Winstanley¹).
- (d) 50 stations between 10° and 20° N, 1953–1972, using August to September on July.
- (e) 5 stations between 11° and 14° N, 1905–1973, using August to September on July.

variation for June rainfall on the southern edge of the Sahel is about 50% but for the total rainfall it is only about 20%.

The simplest predictor would be the regression of July–

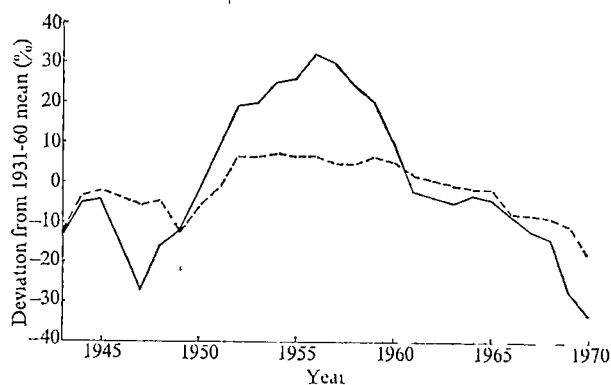


Fig 1 5-yr running means of annual rainfall as percentage of 1931–60 mean for five stations with mean latitude 18° 28' N (ref. 2) (—), and for the five stations used here with mean latitude 12° 48' N (---).

Table 2 Contingency table for July and August–September rainfall based on 5 stations between 11° N and 14° N for the period 1905–73

		August–September		
		Wet	Normal	Dry
July	Wet	8	11	4
	Normal	11	4	8
	Dry	4	8	11

$$\chi^2 = 9.65 \quad P < 0.05$$

Table 3 Correlation coefficients between Niamey 700 mbar mean monthly geopotentials and mean rainfall for five stations between 11° N and 14° N, 1953–72 (probability in brackets)

	June	July	Monthly and seasonal rainfall		July and August	July to September	August and September
			August	September			
Niamey 700 mbar geopotential							
June	–0.353 (20%)	–0.316 (20%)	–0.445 (6%)	–0.305	–0.475 (5%)	–0.317 (20%)	–0.200
July		–0.464 (5%)	–0.637 (1%)	–0.183			–0.443 (6%)
August			–0.781 (0.1%)	–0.008			
September				–0.053			

September rainfall on June rainfall. This regression cannot be reconstructed from Winstanley's graph. But an examination of 50 stations in the same area and for the same period, 1953–72, showed no statistically significant correlation (Table 1). If June rainfall is included in the dependent variable there is a linear correlation, similar, to, but statistically less significant than, that calculated by Winstanley¹. Individual monthly rainfalls were not significantly correlated except for July and August ($r = 0.44$, $P < 0.06$). The regression of August and September rainfall on July rainfall was also statistically significant (Table 1).

A similar analysis for a longer period can be made by using fewer stations. For five stations with relatively long records (Kano, Maiduguri, Sokoto, Niamey and Zinder) departures of rainfall from the 1931–60 normals were calculated and averaged for each year for which records were available in the period 1905–73. The departures at these stations, which are on the southern edge of the Sahel, are similar in pattern to those calculated for five more northerly stations² (Fig. 1). For these southern stations, July and August rainfall ($r = 0.23$, $P < 0.05$) and August and September rainfall ($r = 0.29$, $P < 0.02$) are the only pairs of months to show significant correlation.

The August and September rainfall total is significantly correlated with July rainfall (Table 1). The confidence limits for this regression are large and a useful summary of the observations is obtained by dividing them into terciles and constructing a 3 × 3 contingency table (Table 2). The use of a contingency table emphasises that only a general indication of the amount of rainfall to come can be given. A precise forecast cannot be made.

These results show that West African rainfall has some seasonal persistence, at least from July onwards. Another effect which is probably related was shown by Delsi³ who examined mean monthly geopotentials over the Sudan and West Africa. He found that the mean monthly 700 mbar geopotential anomaly in both areas tended to persist from June until August. Persistence was less evident for earlier months. He also investigated the relationship between the June 700 mbar geopotential anomaly at Khartoum and seasonal (July and August) rainfall for the Sudan for the years 1953 to 1968. The correlation coefficient with rainfall amount was -0.49 ($P < 0.06$) and with number of days with greater than 10 mm of rain was -0.65 ($P < 0.01$).

We have examined the relationships between the Niamey 700 mbar mean monthly geopotential and the average rainfall for the five stations over the period 1955–73. The geopotentials in successive months, between June and September are significantly correlated with correlation coefficients greater than 0.8 ($P < 0.001$). Correlation coefficients between the geopotentials and monthly and seasonal rainfalls are given (Table 3).

For West Africa, seasonal persistence is apparent in the 700 mbar geopotential anomaly from June to September and in rainfall from July to September. As a possible predictor the variations of geopotential in the region itself are likely to be more appropriate than those at 40° N quoted in a general way by Winstanley² or Bryson⁴. Useful seasonal forecasts seem possible. We plan to test such forecasts further in cooperation with the Meteorological Office.

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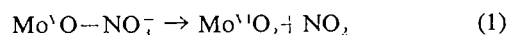
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Possible mechanism for discrimination between nitrate and nitrite by nitrate reductases

RECENT studies¹ have been concerned with the mechanism of redox reactions between nitrate ions and simple mononuclear oxomolybdenum(V) (Mo^{VO}) complexes such as $[\text{MoOCl}_2(\text{OPPh})_2]$ to determine the function of the molybdenum centre in the nitrate reductase enzymes. In the presence of excess nitrate, the following reaction stoichiometry is obtained

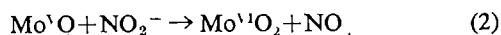


Kinetic studies using stopped-flow techniques identified three stages in these reactions: (1) the loss of the ligand *trans* to the oxo-group of the Mo^{VO} complex followed by rapid, non-rate-determining coordination of nitrate at the vacated site, (2) a rearrangement of the Mo^{VO} -nitrate complex to produce a geometry suitable for rapid electron transfer resulting in the formation of NO_2^- and a *cis*-dioxomolybdenum^{VI} (Mo^{VIO_2}) complex, (3) ligand substitution(s) at the *cis*- Mo^{VIO_2} centre.

Nitrite is considered to be a better nucleophile than nitrate² and redox potential data³ suggest that nitrite should be an oxidant comparable with nitrate. These facts contrast markedly with the exclusive reduction of nitrate by the nitrate reductase enzymes, even though nitrite seems to be able to coordinate to a $\text{Mo}(\text{V})$ centre in these enzymes⁴. We have therefore examined the mechanism of the reaction between $[\text{MoOCl}_2(\text{OPPh})_2]$ and Et_4NNO_2 in CH_2Cl_2 at temperatures from 0 to 25° C for direct comparison with the nitrate oxidation described above. Frank and Spence⁵ have shown that in aqueous solution, $\text{Mo}(\text{V})$ complexes will quantitatively reduce nitrite to NO and we also found this in our system. The non-aqueous system we used provided new information concerning the mechanism of nitrite reduction by a $\text{Mo}(\text{V})$ centre which, when compared to the corresponding reduction of nitrate, suggests possible

ways in which nitrate reductases discriminate between these oxidants

In the presence of excess nitrite, the reaction between $[\text{MoOCl}_3(\text{OPPh}_3)_2]$ and Et_4NNO_2 proceeds according to equation (2) in three stages,



the mechanism of which seems to resemble closely those described above for the corresponding reaction with nitrate. Consistent with the S_N1 (limiting) mechanism proposed for the first stage of this reaction, the rate-determining step involving the loss of the Ph_3PO molecule *trans* to the oxo-group, has a rate constant for nitrite substitution at 25°C of $52 \pm 2 \text{ s}^{-1}$ which is very similar to that obtained ($40 \pm 1 \text{ s}^{-1}$) for the corresponding nitrate substitution. Kinetic data obtained for these substitutions, carried out in the presence of an excess of Ph_3PO , show that the nucleophilicity of these ligands towards the five coordinate intermediate $[\text{MoOCl}_3(\text{OPPh}_3)]^-$ varies in the order $\text{NO}_2^- > \text{NO}_3^- \gg \text{Ph}_3\text{PO}$ as 47 : 31 : 1. This difference between the affinity of the two anions for the Mo(V) centre, together with the general preference for nitrite to function as an N-donor ligand⁶ support a tentative assignment of $[\text{MoOCl}_3(\text{OPPh}_3)(\text{NO}_2)]^-$ as the nitro-complex *a* (Fig 1) rather than the corresponding nitrito-complex. The kinetic data obtained for the second stage of the reaction between $[\text{MoOCl}_3(\text{OPPh}_3)_2]$ and Et_4NNO_2 indicate that the rate of this process (k_2) is independent of the concentrations of Mo^{V} , Et_4NNO_2 and Ph_3PO . At 25°C $k_2 = 2.6 \pm 0.5 \times 10^{-2} \text{ s}^{-1}$ and the process is considered to involve the intramolecular rearrangement of *a* to *b* (Fig 1), a process similar to that suggested¹ for the corresponding stage of the nitrate reaction although this proceeds at a much faster rate, $k_2 (25^\circ\text{C}) = 1.0 \pm 0.2 \text{ s}^{-1}$. A comparison of the ultraviolet spectra obtained (C D G, P M Boorman, P Lambert, F E M, and V I Routledge, unpublished) for Mo(V) and Mo(VI) complexes suggests that the final product of the second stage of the nitrite reaction is a Mo(VI) species. Therefore, after the rearrangement k_2 , a fast electron transfer process, $k_{\text{et}} \geq 5 \times 10^{-2} \text{ s}^{-1}$ at 25°C , is considered to occur, producing *c* (Fig 1) plus NO . The latter was collected as a gas, identified by infrared spectroscopy⁷ and quantitatively estimated by condensation in to acidified KMnO_4 solutions, followed by back titration with Fe(II) (ref 8). The yields of NO were 77–105% of those predicted from equation (2) based on Mo(V) . The changes in the absorbance spectrum

Fig 1 Simple mononuclear Mo^{VO} complexes. Intramolecular reorganisation during nitrate and nitrite reductions. For explanation, see text

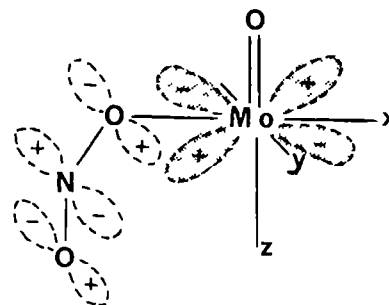
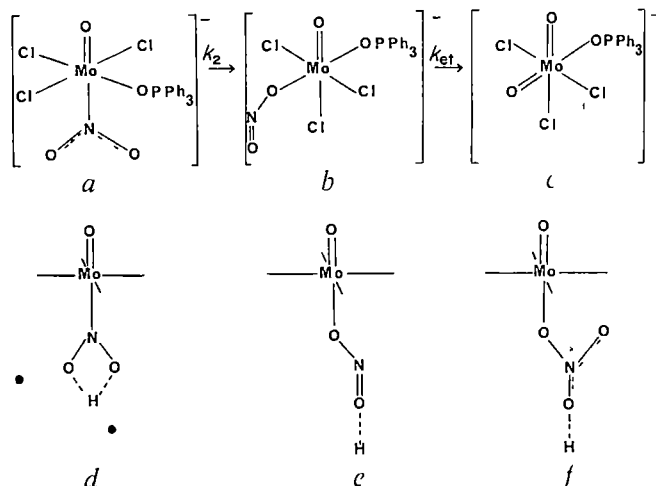


Fig 2 Desired orbital overlap for reduction of nitrite to NO by the Mo^{VO} centre

observed during the third stage of the reaction between $[\text{MoOCl}_3(\text{OPPh}_3)_2]$ and Et_4NNO_2 were consistent with ligand substitution processes of Mo(VI) complexes

The results of this and the previous study¹ suggest that electron- and oxygen-transfer processes between Mo(V) and nitrogen oxanions are interrelated and require a specific atomic arrangement of the reactants. The criteria developed¹ for nitrate oxidation of a Mo(V) centre seem to apply equally well to nitrite oxidation of this entity. Molecular orbital calculations⁸ for the nitrite ion indicate that the π^* orbital is the lowest energy virtual orbital. Therefore, the most favourable route for electron transfer would be expected to occur when this orbital overlaps with the highest energy (partially filled) orbital on the metal, $4d_{xy}$ (ref 10 and C D G, I H Hillier, and F E M, unpublished), providing that electron transfer can lead to the ready formation¹¹ of stable Mo(VI) and N(II) species. Such criteria seem to be exclusive to the arrangement where a nitrito-group is bound to Mo(V) by way of one oxygen atom coordinated *cis* to the oxo-group (Fig 2). This atomic arrangement affords both the desired orbital overlap together with a route for the ready formation of a *cis*- $\text{Mo}^{\text{VI}}\text{O}_2$ moiety and NO .

If the reactive site for nitrate reduction in the nitrate reductases is a mononuclear Mo^{VO} (or $\text{Mo}^{\text{IV}}\text{O}$) centre the site for the easiest initial binding of the substrate would be expected to be *trans* to the oxo-group. The substrate is probably introduced at this position by favourable hydrogen-bonding to a suitably placed X-H group of the protein, differences in hydrogen bonding perhaps accounting for the different affinities for nitrate exhibited by the various nitrate reductases (E J Hewitt, unpublished). It is unlikely, however, that such a site *trans* to the oxo-group could exercise much selectivity for nitrate over other small anionic ligands in general, and nitrite in particular. The coordination of nitrate, nitrite, and azide to the Mo(V) centre suggested by electron spin resonance⁴ studies is consistent with this view. A clear possibility for selective reduction of nitrate over nitrite, however, does exist if, before electron- and oxygen-transfer, the substrate has to be coordinated to the molybdenum by way of one oxygen atom *cis* to the oxo-group. A comparison of the $[\text{MoOCl}_3(\text{OPPh}_3)_2]$ reductions of nitrate and nitrite shows that such reorganisation proceeds some 38 times faster for the former. We suggest that the rearrangement is relatively difficult for nitrite in this model system because it prefers to coordinate as an N-donor ligand⁶. The intramolecular reorganisation (which presumably involves a nitro- to nitrite-isomerisation, the reverse of that reported¹², followed by (O,O) chelation) required to proceed from *a* to *b* (Fig 1) will be considerably greater than that for the corresponding nitrate-complex (compare fig 1f) which has an oxygen atom more suitably situated for the required attack. In the nitrate reductase enzymes, it is possible that hydro-

gen bonding of the substrate could enhance this discrimination. The process could be made less favourable for nitrite, either by making the *cis* coordination of an oxygen atom of the nitro-ligand more difficult (Fig 1d), or by stabilising nitrito-coordination (such coordination could also be favoured by some steric restrictions at the metal site⁶), in which the uncoordinated oxygen atom is held back from the *cis* sites (Fig 1e). This latter model is favoured over the former since a nitrate-ligand could fit directly into the site of *e* and yet have an oxygen atom poised for attack at a site *cis* to the oxo-group (Fig 1f). In this manner the reduction of nitrate could readily proceed, whereas that of nitrite could not.

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Ubiquinone-O in defensive spray of African millipede

WHEN strongly disturbed, the red-legged millipede *Metiche tanganyicense* Kraus (Spirobolida Trigonulidae) sprays attackers with a mixture of four quinones (Fig 1) Ubiquinone-O, a major component of this defensive spray has not been isolated previously from a natural source.

These millipedes were easily collected as they sought refuge in human habitation during the rainy season at Mombasa, Kenya. When roughly handled, the millipede would propel its orange-red defensive secretion about 40 cm. The secretion tans skin and is quite irritating to a mucous membrane, it gives a positive response for *p*-quinones with rhodanine and aqueous ammonia¹.

To collect the defensive spray each millipede was placed in a glass tube and stimulated electrically until it discharged its secretion. Thereafter the millipede was removed from the tube and any secretion adhering to its body was collected on glass wool. Both the glass tube and the glass wool were washed with ether. Gas chromatographic analysis of this solution indicated that four volatile components were present.

Two of these components were readily identified as the common millipede quinones, toluquinone (Fig 1, I) and 2-methoxy-3-methylbenzoquinone (Fig 1, II)². The infrared spectra, mass spectra and gas chromatographic retention time of these components were identical to those of authentic samples.

The third component gave a positive test for a *p*-quinone with rhodanine and aqueous ammonia¹. Its infrared spectrum in CHCl₃ further substantiates a quinoidal structure, showing a strong carbonyl absorption at 1,654 cm⁻¹ and an olefinic absorption at 1,584 cm⁻¹. A dimethoxybenzoquinone

structure (C₈H₆O₄) is suggested by a mass spectral molecular ion at *m/e* 168. The nuclear magnetic resonance (NMR) spectrum in CDCl₃ (values, δ , for chemical shifts in p.p.m.) confirm this, with two olefinic protons (6.68, singlet) and six methoxy protons (4.03, singlet). As this compound has a melting point of 66-67°C it must be 2,3-dimethoxybenzoquinone (Fig 1, III) (melting point, 66-67°C (ref 3)) the melting points of the 2,5-isomer and the 2,6-isomer are 250°C and 254-255°C respectively⁴. This quinone has been previously characterised in the defensive secretion of the millipede *Uroblaniulus canadensis*⁵.

Finally, the component of longest gas chromatographic retention time which also gave a positive colour test for a *p*-quinone¹, showed infrared absorptions typical of a and an olefinic absorption at 1,601 cm⁻¹ (CHCl₃). A dimethoxymethylbenzoquinone structure (C₉H₁₀O₄) is indicated by the observation of a molecular ion at *m/e* 182. The NMR spectrum (CDCl₃) of this compound confirms this, showing an olefinic proton (6.45, quartet *J*=1.5 Hz), three methoxy protons (4.05, singlet), three methoxy protons (4.02, singlet)

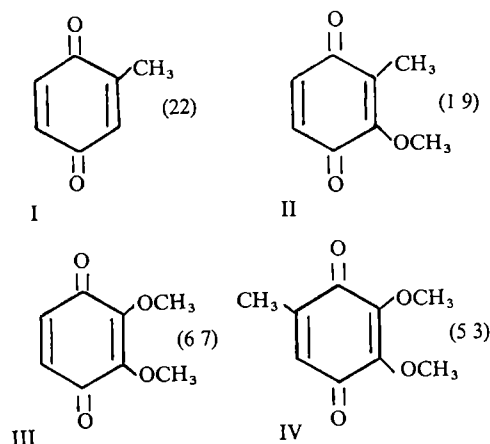


Fig 1 The quinones from *M tanganyicense* with the amount of each (mg) from a millipede (8 g)

and three methyl protons (2.04, doublet *J*=1.5 Hz). As the olefinic proton and the methyl protons are coupled the compound must be 2,3-dimethoxy-5-methylbenzoquinone (Fig 1, IV) (ubiquinone-O). Published NMR⁶ and mass spectra⁷ of IV confirm this assignment.

While spraying of defensive secretions by millipedes is not unknown, discharge from oozing glands is more usual⁸. We have examined anatomically and histologically the glands of *M tanganyicense* and find them to be very similar to those described by Woodring and Blum for the spraying millipede *Orthocricus aboreus*⁹.

Undoubtedly, the red legs of *M tanganyicense* warn predators that it is different from the other millipede common in the area, *Archispirostreptus gigas*, which is entirely dark brown and has oozing glands that secrete a mixture of toluquinone and 2-methoxy-3-methylbenzoquinone¹⁰. The difference between these two millipedes was learned by the dwarf mongoose *Helogale parvula* on its initial encounter with *M tanganyicense*—one spraying was sufficient to discourage further attacks on this millipede (P Robins, unpublished). *A gigas*, which does not spray its secretion, was readily eaten by the same mongoose. On the other hand, the elephant shrew *Rhynchocyon chrysopygus*, which inhabits the coastal forests of Kenya, eats *M tanganyicense* and does not seem bothered by its defensive secretion (G Rathbun, unpublished).

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Piezoelectric properties of bone as functions of moisture content

FUKADA and Yasuda¹ showed that dry bone is piezoelectric in the classic sense, that is, mechanical stress produces polarisation (direct effect) and application of an electric field produces strain (converse effect)² The possibility that the mechanism for osteogenesis is electrical³ has aroused interest in an investigation of such effects in the more nearly physiological condition represented by wet bone Recent developments in electrical stimulation of fracture healing^{4,5} have further emphasised the importance of characterising the piezoelectric properties of wet bone As the piezoelectricity of wet collagen⁶ decreases to zero at a moisture content equal to 45% of the dry weight (which corresponds to almost 100% humidity)⁷, there is some doubt as to whether wet bone could be piezoelectric Observations of voltages in wet bone under stress, have been made under conditions giving such ambiguous results that they suggested alternative concepts to that of classic piezoelectricity⁸⁻¹¹ In view of the confusion about the nature of the voltage developed when wet bone is stressed, we have used a simple longitudinal stress system with sinusoidally varying drive, to measure both converse and direct piezoelectric coefficients of bone as functions of humidity (and ultimately of moisture content) The results show unambiguously that wet bone behaves as a piezoelectric material

Bone was cut from adult human femur, and stored in Ringer solution at 3°C for several weeks Samples (4.2 mm³) were cut in the two orientations shown in Fig 1, that is parallel to the three principal axes, and rotated 45° about the x axis Silver epoxy electrodes were attached to the endosteal and periosteal surfaces (perpendicular to the x axis) for the 45° specimens and perpendicular to the bone axis for the unrotated specimens

A test apparatus similar to Fukada's¹² was used with some modifications so that both converse and direct effect could be measured These included additional shielding and loading the bone and quartz outputs with a large capacitor, so that a charge amplifier was used to measure direct effect A bone specimen was clamped together with a quartz crystal cube and a ceramic disk of high piezoelectric coefficient (PZT5) The PZT5 ceramic served to drive the system for the direct effect, and acted as the strain detector for the converse effect The quartz crystal was used as a standard

The frequency (in the range 3,200 to 5,600 Hz) was chosen so that the system was at resonance, to increase the sensitivity and to reduce the effect of pickup By keeping the test apparatus in a sealed chamber containing an appropriate saturated salt solution, and immersing the chamber in a constant temperature water bath, both temperature and humidity were controlled One week was allowed for equilibration at each humidity

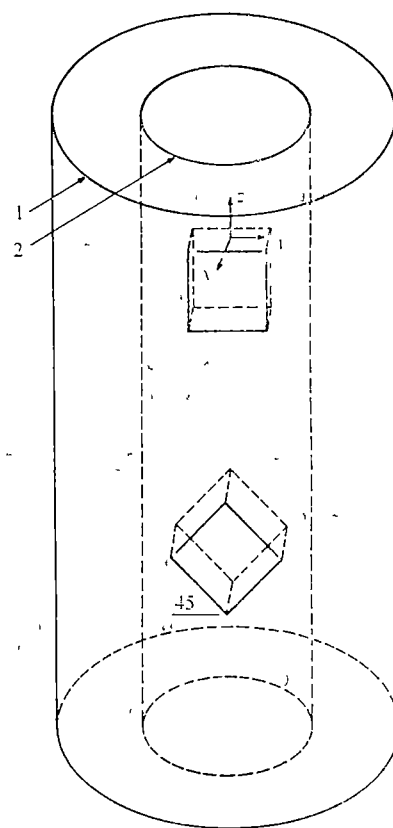


Fig 1 Location of bone specimens with respect to femur 1, Periosteal surface, 2, endosteal surface

From work on dry bone¹, we know that the principal piezoelectric coefficient is the quantity d_{14} , which involves coupling between an electric field in the x direction and a shear strain in the yz plane The coefficient d_{14} is most easily obtained from the specimen rotated 45° about the x axis of the bone (Fig 1), using longitudinal strain Experiments carried out with the sample cut parallel to the x axis yielded the coefficient d_{31} (that is, electric field or polarisation in the z direction)

The variation of d_{14} with humidity (obtained from both converse and direct effects) is shown in Fig 2 The agreement obtained between the measurements of converse effect and direct effect is quite good, we believe the small systematic disagreement is experimental in origin Comparison of data taken in the direction of increasing humidity with those taken with decreasing humidity shows, however, that the measurements display a hysteresis effect, that is, the piezoelectric coefficient is a function of previous hydration history as well as of relative humidity. Recognis-

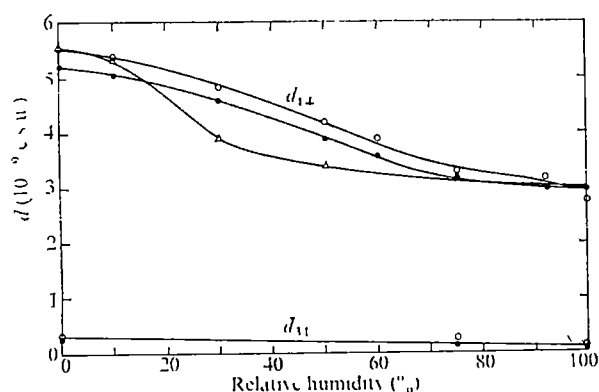


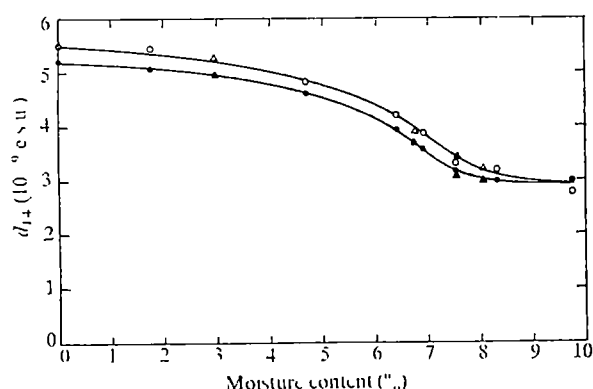
Fig 2 Piezoelectric coefficients of bone as functions of relative humidity measured at 5,600 Hz d_{14} , Converse effect, dry to wet (○), direct effect, dry to wet (●), converse effect, wet to dry (△) d_{31} , Converse effect, dry to wet (○) direct effect, dry to wet (●)

ing that moisture content (that is, weight of water per unit weight of dry bone) represents a more fundamental variable than humidity, we determined the moisture content of similar samples of bone as a function of relative humidity by direct weight-change measurements over several wetting and drying cycles¹³. These measurements were used to convert the piezoelectric data from functions of humidity to functions of moisture content. The piezoelectric coefficient d_{14} is shown plotted in this way in Fig 3, which shows that as a function of moisture content the data give single-valued behaviour, with no hysteresis, that is, the results are independent of past history (Figure 3 also includes data for the direct effect in the direction of decreasing humidity, which have been omitted from Fig 2 for the sake of clarity).

From a comparison of the present results with the variation of d_{14} of collagen with moisture content, it follows that at 100% humidity the collagen in bone must have a moisture content of 12%, only slightly higher than the average moisture content of bone itself. The moisture uptake of free collagen⁶ and of collagen in bone differs probably because the latter is intimately bonded to the mineral phase and is not free to swell.

Using a specimen cut parallel to the bone axes, we measured the piezoelectric coefficient d_{31} . As Fig 2 shows, the values of d_{31} are an order of magnitude lower than d_{14} , but decrease with increasing moisture content in the same way. This result is in direct contradiction to the work of Anderson and Eriksson⁹ who observed increasing values of d_{31} for wet bone, and attributed these results to streaming potentials. Our results show no evidence to support the streaming-potential concept. Rather, it can be stated that for sinusoidal drive at audio frequencies, bone behaves in a classic piezoelectric manner, with good agreement between converse and direct effect, at all humidities.

Fig 3 Coefficient d_{14} of bone as function of moisture content converse effect, dry to wet (○), direct effect, dry to wet (●), converse effect, wet to dry (△), direct effect, wet to dry (▲)



The following paper¹⁴, demonstrating that both bone and tendon are piezoelectric in the hydrated, though frozen, state, is consistent with our findings.

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Piezoelectricity in hydrated frozen bone and tendon

THE piezoelectric property of connective tissue may play a role in regulating the patterns of tissue growth¹. Most piezoelectric measurements, however, deal with dried tissue²⁻⁴. Observations of stress-generated voltages from hydrated connective tissue are generally insufficient to establish that the voltages are of piezoelectric origin, because of complications resulting from streaming potentials and electrode effects⁵⁻⁷. A report of the non-existence of piezoelectricity in hydrated collagen at room temperature⁸ has been criticised⁹. Use of the converse effect is most desirable in studying biological piezoelectricity⁹, but for hydrated tissue the high electrical conductivity of water interferes with the establishment of an electric field inside the sample^{2,4}. We therefore hydrated and then froze our connective tissue samples taking advantage of the reduced conductivity of ice compared with that of water. Our results establish the existence of the piezoelectric effect in bone and tendon under physiological conditions of moisture, but at a non-physiological temperature (-25°C).

We used femurs and Achilles tendons of cows, aged 3 to 4 yr when slaughtered. Samples approximately $10 \times 5 \times 3$ mm, orientated to display the piezoelectric coefficient d_{14} , were prepared and measured by the converse effect.

Measurements on dry bone and tendon were made at 24°C after drying the samples at 100°C for 24 h. Samples were fully hydrated by immersing in saline at 24°C for 24 h then after hydration, they were frozen at -25°C for 24 h and then measured at -25°C . The small dimensional changes in bone following hydration were ignored, whereas the corresponding changes for tendon were not negligible and therefore the dimensions of the frozen tendon samples were used to compute their hydrated piezoelectric coefficients.

The results (Table 1) show that hydrated frozen bone and tendon are piezoelectric. The effect of both freezing and hydrating is to reduce the magnitude of d_{14} by about a factor of two for bone and a factor of eight for tendon. About half of the decrease for tendon can be accounted for on the basis of the volume increase which accompanies hydration.

Whatever structural changes result from the incorporation

Table 1 Piezoelectric coefficients (d_{14}^*) and standard deviations of bovine bone and tendon

	Dry (at 24° C)	Hydrated (at -25° C)
Bone	5.45 ± 0.82 (22)	2.9 ± 0.6 (7)
Tendon	86.6 ± 18.4 (4)	10.2 ± 2.8 (7)

Numbers in parentheses indicate number of samples measured
 $\times 10^{-9}$ cgs esu

of water by collagen, the piezoelectric property is relatively unaffected at -25° C. In view of the marked stability of collagen as determined by piezoelectric measurements^{4,10}, it seems unlikely that an increase to physiological temperatures would produce structural changes so drastic as to destroy the piezoelectric property. The preceding paper¹¹ presents evidence which supports this contention.

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Ultraviolet light activation of insect nuclear polyhedrosis virus

IRRADIATION within the ultraviolet (190-380 nm) range is an effective means of virus inactivation. We report the effects of short exposures of ultraviolet light at 320 nm on *Autographa californica* (Lepidoptera) nuclear polyhedrosis virus. In this case, ultraviolet light has an activating effect on the virus produced *in vitro*.

An aqueous suspension of *Trichoplusia ni* nuclear polyhedrosis virus inclusion bodies seems to be completely inactivated when exposed to 253.7 nm ultraviolet light at an incident distance of 15 cm for 1 min¹. Gudauska and Canerday² exposed partially purified suspensions of *T. ni* nuclear polyhedrosis virus and *Heliothis* nuclear polyhedrosis virus to 253.7 nm ultraviolet light at a distance of 2-5 cm, and reported apparent total inactivation of the *Heliothis* virus after 5 min at 2 cm and almost total inactivation of the *Trichoplusia* virus by 10 min at 2 cm. They noted a decrease in ultraviolet inactivation as the incident distance increased. Bullock *et al.*³ exposed purified suspensions of *Heliothis* polyhedra to wavelengths of 253.7, 307.5 and 364 nm at a distance of 10.2 cm, and found significant inactivation of virus in the 253.7 and 307.5 nm range. They suggested that at 364 nm some virus reactivation may have occurred. Witt⁴ studied the photochemistry of *Galleria mellonella* nuclear polyhedrosis virus and found that non-occluded nucleocapsid suspensions were inactivated when exposed to ultraviolet light of 253.7 nm at 50 cm and 320.0 nm at 25 cm. The near ultraviolet (320 nm) did not increase virus activity.

Immediately after exposure of the virus samples (prepared as in Fig. 1) to ultraviolet light, larval inoculation was performed. For each sample, twenty larvae were intracoelomically inoculated with 0.5 μ l per larva. Twenty larvae were injected with a blank sample containing only distilled water. The *in vitro* plaque assay of the irradiated suspensions showed that,

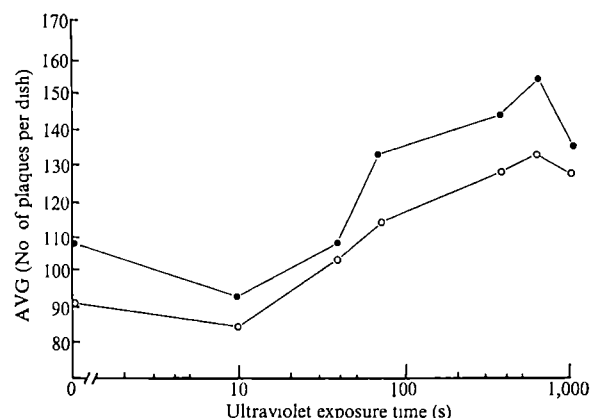
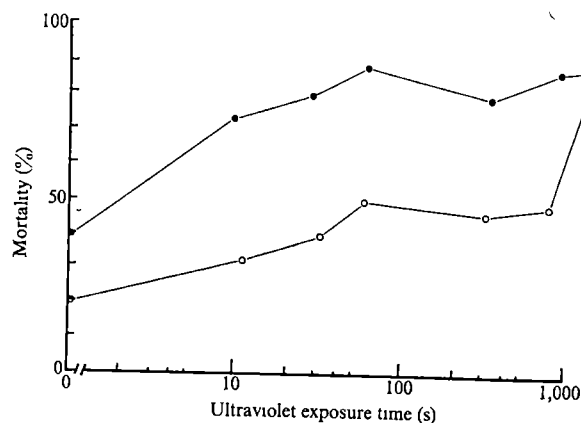


Fig. 1 Plaque assay of *A. californica* nuclear polyhedrosis virus, irradiated with ultraviolet (UV) light on TN-368 cells. Each line represents a replication. Each dot is the average of two plaque counts. Polyhedra-free nucleocapsid suspensions were assayed *in vitro* using a plaque assay technique⁶ on the *Trichoplusia ni* (TN-368) cell line. The suspensions were also bioassayed on 7-d-old *T. ni* larvae. The viral inoculum was obtained by infecting the TN-368 cell monolayers with *A. californica* nuclear polyhedrosis virus. Cells from these infected monolayers were centrifuged at 164g, resuspended and washed twice in Hanks' phosphate buffered saline⁷. The saline was drawn off and the cells resuspended in distilled water. This procedure lysed the cells and the suspension was spun at 1500g for 20 min. The supernatant was filtered through a 0.45 μ m Millipore filter. This was the stock preparation. Plaque assays were performed on serial tenfold dilutions of this preparation for quantitating plaque forming units (PFU). Similarly, the LD₅₀ of the virus preparation was determined by bioassaying serial tenfold dilutions by injection into 7-d-old *T. ni* larvae. The virus preparation was diluted to approximately 10⁴ PFU ml⁻¹ in sterile distilled water. Aliquots (1 ml) were pipetted into depressions (2 cm diameter) of plastic depression trays (Limbco Chemical Co., Inc.) and exposed to two General Electric BLB fluorescent black lights (output 15 W at 320.0 nm) at 22 cm. The samples were exposed for time periods of 0 to 17 min and were gently agitated during exposure. After exposure, the suspensions were diluted to the titre which would allow the assays to be read.

rather than inactivation, virus activation seems to have occurred (Fig. 1). A comparison of plaque numbers of the three shortest irradiation periods (0, 10 and 30 s) with the three longest irradiation periods (300, 600 and 1,020 s) using an unpaired *t* test⁸ revealed a difference between means that was significant at the 98% level ($t = 2.89$). An activation mechanism would seem to be responsible for this significant increase in plaque forming units. The results of the larval bioassay showed a marked increase in virus activity as ultraviolet exposure time increased (Fig. 2). This represents approximately a 200-fold increase in virus activity when the LD₅₀ values are calculated.

Fig. 2 Larval (8-d-old *Trichoplusia ni*) bioassay of *A. californica* nuclear polyhedrosis virus irradiated with ultraviolet light. Each line represents a replication.



These data show that near ultraviolet light has an activating effect on *A. californica* nuclear polyhedrosis virus produced *in vitro*. The two assays demonstrate that the effect is related to radiation dosage. The larval bioassay suggested that most activation occurred during the first 60 s and the plaque assay that the effect began at 60 s and lasted for 420 s. This may result from differences in the sensitivities of the two assay systems.

Ultraviolet light enhanced by reactivation and photo-reactivation occurs in mammalian viruses⁹, avian viruses¹⁰ as well as plant viruses¹¹. We present the first account of near-ultraviolet light induced activation of an insect nuclear polyhedrosis virus. Further investigation will be needed before the mechanism involved in this activation can be determined.

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Olfactory response in excitable protoplasmic droplet and internodal cell of *Nitella*

IN higher vertebrates, olfactory reception takes place at the receptor cell in the olfactory epithelium. The elicitation of neural responses caused by odorants is not restricted to the olfactory organs, other excitable systems (for example, the trigeminal nerve and vomeronasal organ of the tortoise^{1,2}, ganglion cell³ of *Helix* and contact chemoreceptor of the fly⁴) are also to respond to odorants. Excitable membranes in general probably respond to odorants, and the processes of olfactory reception and of excitation of a membrane must be closely related. The interaction between odorants and an excitable membrane which is basic to the molecular process of olfactory stimulation, has not been investigated with this relationship in mind, because of the small size of the olfactory cells and of the excitable systems mentioned. This factor has prevented the recording of the intracellular receptor potential in response to odorants. To clarify the physicochemical mechanism underlying the olfactory reception, we have therefore used the protoplasmic droplet and internodal cell of *Nitella* to investigate the effect of odorants on the electrical properties of an excitable membrane. Apart from its size, the protoplasmic droplet has the advantage of having a functional membrane contiguous with the external solution⁵⁻⁷.

An internodal cell of *Nitella flexilis*, immersed in a solution containing 0.5 mM CaCl₂ and 300 mM mannitol with no univalent cation species, has a resting potential of about -100 mV and an action potential of 50-60 mV (about 30 s long) after electrical stimulation. The excitable protoplasmic droplet was prepared as previously described^{8,9}. The internodal cell was amputated and the effused protoplasmic droplet allowed to stand in a salt solution (0.5 mM NaNO₃, 0.5 mM KNO₃, 1 mM CaCl₂, and 2 mM MgCl₂)

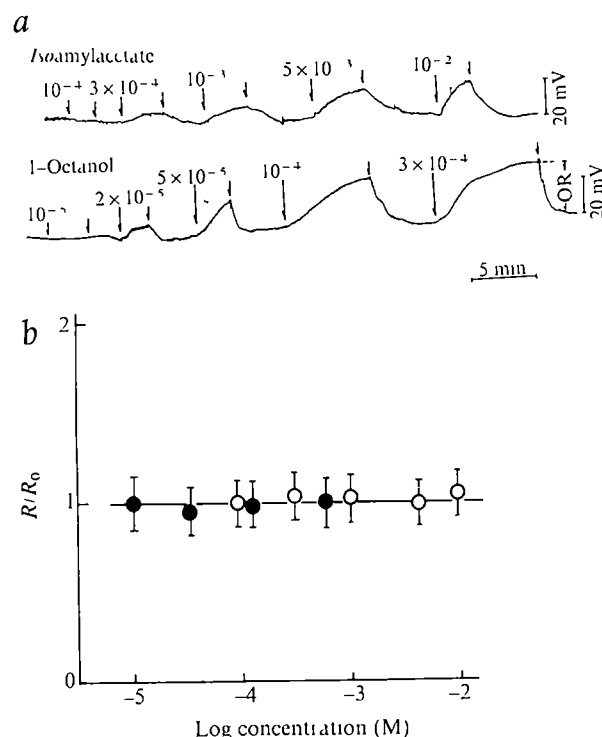


Fig 1 Variations in the membrane potential and the electrical resistance observed for a protoplasmic droplet in response to various concentrations of isoamylacetate (○) and 1-octanol (●). Traces illustrate the time-courses of potential response, arrows (numbered) show the time of odorant application, and the following arrow (no number) shows the time of odorant removal. The number indicates the concentration of odorant applied in mol l⁻¹. Graph shows relative electrical resistance (R/R_0) as a function of concentration of the odorants, where R_0 stands for the resistance of the surface membrane of the droplet in the resting state. Temperature, $20 \pm 1^\circ \text{C}$.

After the membrane potential had reached a steady value of about -90 mV, at which the droplet became electrically excitable, various concentrations of odorants were applied to the external medium. Intracellular potentials were recorded using a microelectrode. This resulted in a depolarisation of the membrane potential, recorded using a microelectrode, when the concentration exceeded a certain value.

Figure 1a represents two examples of the depolarisation when various concentrations of 1-octanol and isoamylacetate were applied to the droplet. The potential varied continuously with time and approached a steady value. Deviation of the steady potential from the original level is referred to as the olfactory response (OR), and the resting potential could be recovered after removal of the odorants. The membrane resistance of the protoplasmic droplet decreases markedly during excitation, as in squid giant axons^{8,9}. In contrast to electrical excitation, the membrane resistance of the droplet remained practically constant when the droplet was depolarised in response to odorants. Figure 1b shows the relative values of the electric resistance (R/R_0) of the surface membrane as a function of the concentration of the odorants, where R_0 stands for the membrane resistance of the droplet in the resting state.

When the odorants were applied to the internodal cell, the membrane potential varied in a complicated manner. Typical examples of the potential response are shown in Fig 2. The potential varied sharply around 60 mV when the odorant was applied, decreasing slightly within a few seconds. It changed again with time, passed through a maximum and approached a steady value, returning to its original level when the odorant was removed from the

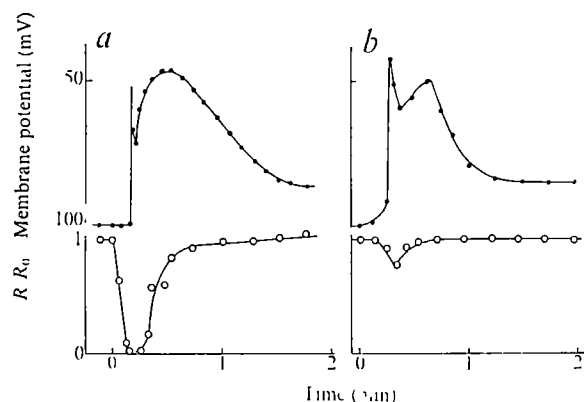
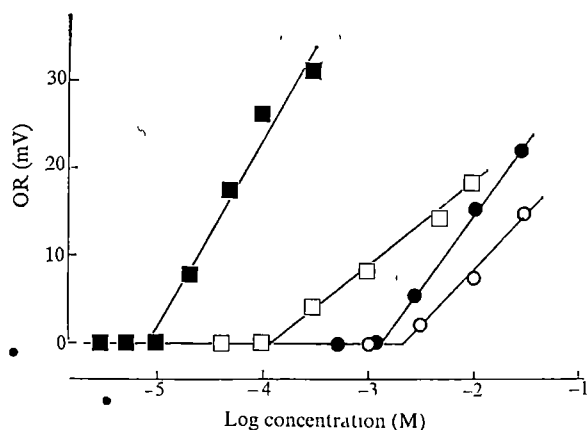


Fig 2 Time-courses of variations in the membrane potential and the relative electrical resistance for an internodal cell in response to *a*, 3×10^{-3} M isoamylacetate and *b*, 5×10^{-4} M 1-octanol Temperature, 23°C

medium The height of the first peak of the potential variation was independent of the odorant concentration, whereas that of the second peak and the final steady value of the membrane potential depended on the odorant concentration Simultaneous measurements of the electrical resistance showed that the first peak of the membrane potential was accompanied by a marked decrease in the membrane resistance, as in the case of electrical excitation This decrease recovered within 30 s, and the resistance was approximately constant during the following variation in the membrane potential (Fig 2) The steady value for the electrical resistance of the membrane during olfactory response was almost the same as that in the resting state When the internodal cell is not electrically excitable, no potential response is elicited after application of odorants

Figure 3 shows the variations in the membrane potential at the steady value, OR, for the protoplasmic droplet, plotted as a function of odorant concentration (C) in the medium The membrane potential did not change until the concentration of odorant reached a threshold value (C_{th}), OR increased almost linearly with increase in odorant concentration above C_{th} when OR was plotted against $\log C$ In the internodal cell, this linear relationship similarly held for the height of the second peak of the membrane potential change The value of C_{th} determined from OR $\log C$ relationships for the protoplasmic droplet is in agreement with the olfactory threshold (T) for human¹⁰, and plots of $\log T$ against $\log C_{th}$ fall on a straight line of unit slope (Fig 4) A similar unit slope relationship was obtained with the

Fig 3 Deviation of the steady potential from the original level (OR) in response to odorants plotted against \log odorant concentration (C) for the protoplasmic droplet Experimental conditions as in Fig 1 ■, 1-Octanol, □, isoamylacetate, ○, ethyl ether, ●, 1-butanol



threshold observed in the internodal cell The lack of variation in electrical resistance of the membrane both for the internodal cell and for the protoplasmic droplet during the olfactory response induced by 1-octanol or isoamylacetate seems to be difficult to interpret in terms of the puncturing theory¹¹, in which an odorant is assumed to puncture the olfactory receptor membrane temporarily, leading to a change in the ionic permeability of the membrane The change in the membrane potential induced by odorants, at least in the above cases, may be attributed to a change in the potential produced at the membrane-solution interface as discussed in a recent study of taste reception^{12, 11} Further, the olfactory receptor of the box turtle responds to odorants

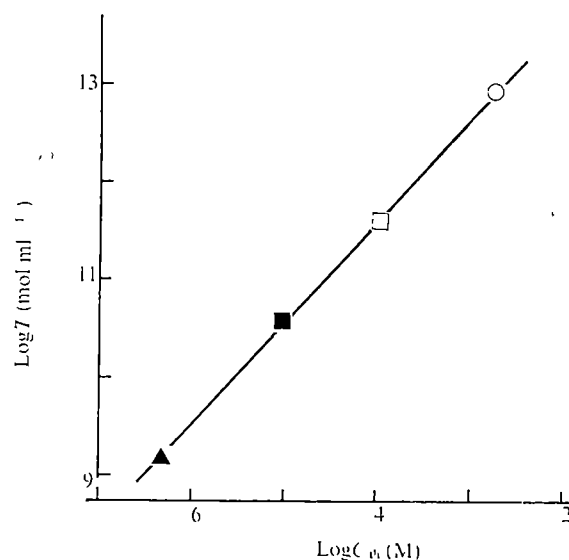


Fig 4 Linear relationship with unit slope between $\log T$ and $\log C_{th}$, where C_{th} stands for the threshold of olfactory response for the protoplasmic droplet in *Nitella* and T represents the human olfactory threshold¹⁰ Symbols as in Fig 3, plus skatol (▲)

even when a Na^+ -free solution is placed on the olfactory epithelium, and a removal of Ca^{2+} from the olfactory epithelium brings about an increase in spontaneous firing of the olfactory nerve¹⁴ The thresholds of the protoplasmic droplet to odorants were markedly lowered by a decrease in Ca^{2+} concentration in the medium For example, the threshold for 1-octanol was lowered by a factor of 10^{-2} with a tenfold decrease in Ca^{2+} concentration This decrease also increases the instability of the surface membrane of the droplet^{9, 15} It is therefore not unreasonable to consider that the extremely high sensitivity of the olfactory receptor cell to odorants may be attributed to the instability of the receptor membrane

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Failure of exogenous gonadotrophin controlled ovulation to cause digit abnormalities in mice

GONADOTROPHINS have been used for many years in laboratory work to induce ovulation in animals at specific convenient times¹⁻⁵. An injection of pregnant mare serum (PMS) induces follicular growth, and if this is followed by an injection of human chorionic gonadotrophin (HCG) the mature follicles are ovulated at a predictable time. There are a few suggestions in the literature that this practice may lead to abnormalities in the offspring of the treated females⁶⁻⁸. In particular, Elbling⁹ reported that abnormalities in the digits and an altered sex ratio were found in litters born to gonadotrophin-treated female mice. As the practice of inducing ovulation by these means is widespread in research and is of practical importance in swine husbandry, we felt that this claim against PMS and HCG should be evaluated. We therefore repeated part of the experiment performed by Elbling⁹, with slight modifications, in that we did not study postnatal survival but observed digit normality, sex ratio and litter size in two stocks of mice in an effort to detect the influence that different genetic backgrounds may have in the response to exogenous gonadotrophins. We found no abnormalities in the digits or altered sex ratio in the offspring.

were sexed by making a mid-ventral incision and locating the gonads. Digits from all four paws were observed and if an abnormality was thought to be present, the paw was severed from the limb for study. It was then fixed, dehydrated, embedded in paraffin and sectioned with a rotary microtome at 8 μ m. Slides from the sample were stained with haematoxylin and eosin. The presence of all metacarpals and metatarsals was used as the criterion for digit normality together with a visual comparison made with digits from sections of the corresponding normal paw of the same or other animals.

Of the 640 offspring from 45 litters observed in the gonadotrophin-injected group, none showed missing digits on any limb. One foetus displayed an abnormal right hind limb which appeared to be lagging behind in development although all digits were found to be present on histological examination. In the control group of 266 foetuses from 24 litters, no digit or limb abnormalities were noted. Neither stock of mice, ICR or J, when compared with the controls for that stock by a χ^2 test, showed a significant difference in sex ratios. There was a significant increase in litter size in both gonadotrophin-treated stocks. This was not unexpected, however, and may be a result of superovulation caused by the gonadotrophin injections.

Both treated stocks showed an increase in prenatal mortality when compared with the controls. The ICR treatment group averaged 2.5 degenerations per litter compared with an average of 0.125 in the ICR controls. All of the ICR losses were judged to be early embryos because they were quite small and were being resorbed. The J treatment group showed an average of 0.54 prenatal deaths compared with 0.375 in the J controls. Of a total of 18 J treatment prenatal deaths, only five were late foetal deaths with the remaining 13 classified as resorbing early embryos.

Early embryonic mortality has been previously attributed to PMS-HCG controlled ovulation in mice by McLaren and

Table 1 Summary of data on reproduction of gonadotrophin-programmed mice

Stock	Treatment		Control	
	ICR	J	ICR	J
No. of litters	12	33	8	16
Total no. of foetuses	188	452	91	175
Average litter size*	15.67 \pm 2.77†	13.7 \pm 0.9†	11.38 \pm 1.48	10.94 \pm 0.82
No. of degenerating foetuses	30	18	3	2
Sex ratio*				
Male (%)	56.9	52.9	59.3	49.1
Female (%)	43.1	47.1	40.7	50.9

*Includes late degenerating foetuses but not degenerating embryos.

†Significantly different from control ($P < 0.05$).

Two random bred stocks of mice, ICR and J, were obtained from a colony maintained at Purdue University. The J stock was developed from four-way crosses between inbred lines LP/J, SJL/J, BALB/cJ, and C57BL/6J. The ICR mice originated from stocks with large litter size developed at the Institute of Cancer Research, Philadelphia, Pennsylvania. All female mice had borne one litter through natural mating and none had previously been exposed to any type of drug. Gonadotrophins were dissolved in 0.85% sterile NaCl solution such that each injection of 0.25 ml delivered 5 IU of PMS (NIAMDD, Bethesda, Maryland) or HCG (Ayerst, Chicago, Illinois).

Forty-five female mice, 12 ICR and 33 J were programmed to ovulate by intraperitoneal gonadotrophin injections of 5 IU PMS followed 48 h later by 5 IU HCG. Immediately after the second injection, each female was caged overnight with a male from the same stock. Twenty-four female mice, 8 ICR and 16 J, were used as controls. The presence of a copulatory plug (CP) the next morning after mating was used to determine day one of pregnancy. Seventeen days post-CP, the females were killed by cervical dislocation and their pups removed by dissection.

Litter size was determined and degenerating foetuses recorded. Average litter sizes include late foetal deaths but not early degenerating embryos. Using a stereomicroscope, pups

Michie⁹. In their study, using mice from Theiler's Original strain, treated adults had a higher incidence of embryonic mortality from 7.5 to 9 d of development than either treated immature females or controls. The specific mechanisms involved are not clear but several hypotheses have been forwarded⁹. These include maternal hormonal deficiencies, and delays in fertilisation because of improper timing of ovulation in relation to insemination so that 'aged' ova are fertilised. Delays in fertilisation have been shown by Butcher and Fugo¹⁰ to result in chromosome anomalies in rat embryos.

We found no evidence to link gonadotrophin-induced ovulation with digit abnormalities of the offspring, nor was any alteration of the sex ratio observed. This is in disagreement with Elbling⁹ who observed digit abnormalities in 5 out of 32 treated litters and a shift in the sex ratio in favour of females in litters of treated Swiss albino virgin mice.

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Cytochalasin B inhibits stabilisation of adhesions in fast-aggregating cell systems

THE rapid, naturally occurring aggregation shown by limpet haemocytes¹ and mammalian blood platelets^{2,3} is accompanied by production of prominent microspikes^{1,3} and is reversibly inhibited by cytochalasin B (CB)^{4,5}, thus differing from the slower aggregation of dissociated tissue cells⁶⁻⁸. Although CB inhibits aggregation of limpet haemocytes in shaken suspension, preformed aggregates shaken in the presence of CB are not disrupted, suggesting that CB inhibits the formation of stable contacts⁹.

By light microscopy, the first observable effect of CB on limpet haemocytes is collapse of the microspikes, apparently as a result of disruption of their supporting cores. An electron microscope study of the system (our unpublished work) confirms this by showing that CB causes rapid disappearance of the microfilament bundles (Figs 1 and 2) which constitute the core in these, as in other, microspikes⁹. The extreme rapidity and the reversibility of these two effects of CB, both the disappearance of microspikes and the inhibition of aggregation, suggests that these two phenomena may be causally linked. A possible basis for such a link is the theoretical requirement for low diameter projections to establish initial contact between similarly charged cell surfaces^{10,11}. The observation that haemocytes in the presence of CB spread on to a glass surface at a much slower rate than normal⁵, leads to the alternative suggestion that the contacts between colliding cells in shaken suspension are normally stabilised by the rapid spreading of participant cells over each other's surfaces by a microspike-dependent process similar to that by which they spread on to glass, and thus that spreading of microspikeless cells in the presence of CB is too slow to effect this stabilisation. We have attempted to distinguish between these hypotheses by studying the aggregation of limpet haemocytes maintained in prolonged contact in a centrifugal pellet in the presence of CB.

Cells in blood taken from the pallial vein of each of six limpets were submitted to the schedule shown in Fig. 3 as far as counts 3A-D, and in three of these cases, incubation in EDTA-seawater⁶ and counts 4A-D were added to estimate the number of cells adhering to the test tube. Particle numbers (one particle is one cell or a group of contacting cells) were estimated by haemocytometry and expressed as number per tube.

Essentially similar results were obtained from all the experiments, although there was considerable variation between cells taken from individual animals with regard to initial particle density, extent of aggregation and the number of cells lost by attachment to the test tubes. The results of a representative experiment are shown in Table 1. As expected from previous studies^{1,5}, haemocytes pelleted in artificial seawater (tube A) aggregate extensively within 5 min and, following dispersal, show no further aggregation on the shaker, presumably having reached an endpoint. In contrast, the preparations in CB disperse to give high particle numbers after incubation in the pellet for 5 min. With increasing incubation time, the dispersed particle number decreases progressively and by 30 min approaches the

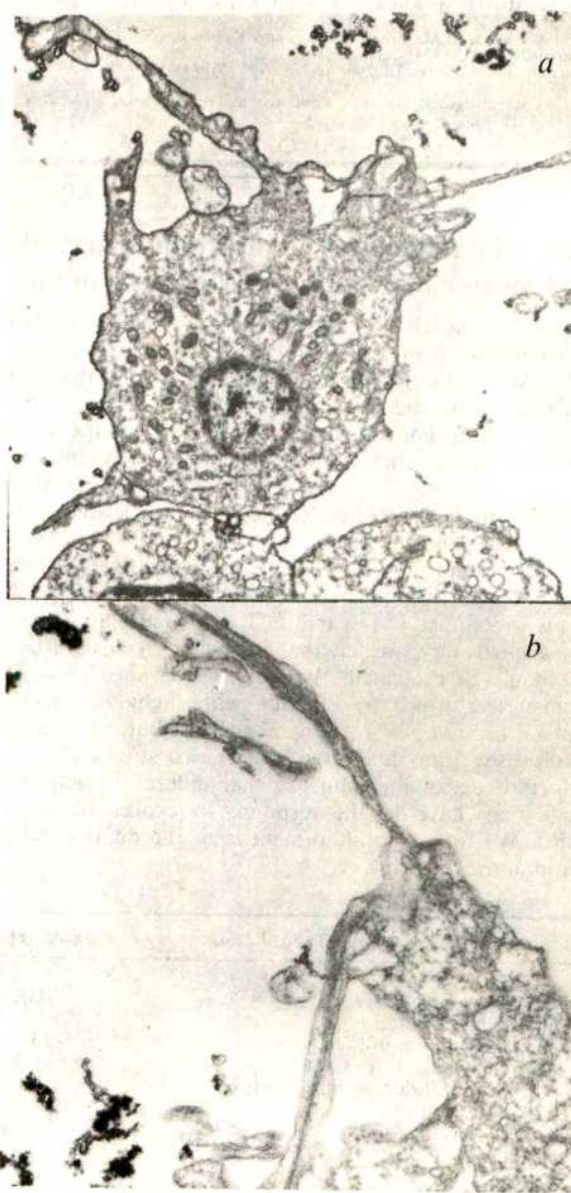


Fig. 1 *a*, Limpet haemocyte fixed from artificial seawater. Note well developed microspikes. ($\times 8,600$). *b*, High power of cell microspikes showing supporting core of microfilament bundles. ($\times 21,500$).

values seen in the artificial seawater control, with large aggregates appearing in the suspensions in the counting chambers. Counts 3B-D confirm that these aggregates are stable and also that no further aggregation occurs in the shaker system. It is evident from counts 4A-D that a negligible proportion of the particles is lost in the form of cells remaining attached to the glass after dispersal of the pellet. Thus it seems that cells maintained in contact in the presence of CB form stable adhesions to one another, but do so more slowly than do cells in artificial seawater.

The demonstration that stable contacts can be formed between CB-treated haemocytes does not preclude the hypothesis that spikes are normally required for the initiation of contact between cells in shaken suspension, for it is possible that the barrier of electrostatic repulsion between microspikeless CB-treated cells is overcome by the pressure of centrifugation. This hypothesis fails, however, to account for the fact that the degree of aggregation increases with incubation time. This observation suggests that haemocytes, in the absence of

Table 1 Effect of cytochalasin B on aggregation of pelleted limpet haemocytes

COUNT 1		$6.6 \pm 0.3 \times 10^5$			
Initial particle no. per tube					
Tube		A	B	C	D
Incubation medium		$1 \text{ mm}^3 \text{ cm}^{-3}$ DMSO in ASW		$0.5 \mu\text{g cm}^{-3}$ CB in ASW	
Incubation times in pellet		5 min	5 min	10 min	30 min
COUNT 2					
Particle no. after dispersal of pellet		$5.7 \pm 0.84 \times 10^4$	$1.93 \pm 0.13 \times 10^5$	$1.68 \pm 0.16 \times 10^5$	$1.02 \pm 0.07 \times 10^5$
Particle loss in pellet (%)		91	71	75	85
P		0.001	0.001	0.001	0.001
COUNT 3					
Particle no. after 5 min on shaker		$4.7 \pm 0.75 \times 10^4$	$1.82 \pm 0.15 \times 10^5$	$1.64 \pm 0.2 \times 10^5$	$1.12 \pm 0.15 \times 10^5$
Particle loss on shaker (%)		17.5	5.7	2.4	-9.8
P		0.4	0.6	0.9 0.8	0.5
COUNT 4					
No. of cells lost by adherence to test tube		$3 \pm 1.5 \times 10^3$	$4 \pm 1.0 \times 10^3$	$6.5 \pm 1.5 \times 10^3$	$4 \pm 1.3 \times 10^3$

Results of a representative experiment following schedule shown in Fig. 3. Counts of particle numbers are expressed per tube. Percentage loss between two preceding counts are shown and the *P* values are calculated from *t* tests on the two preceding counts in each tube. ASW, Artificial seawater.

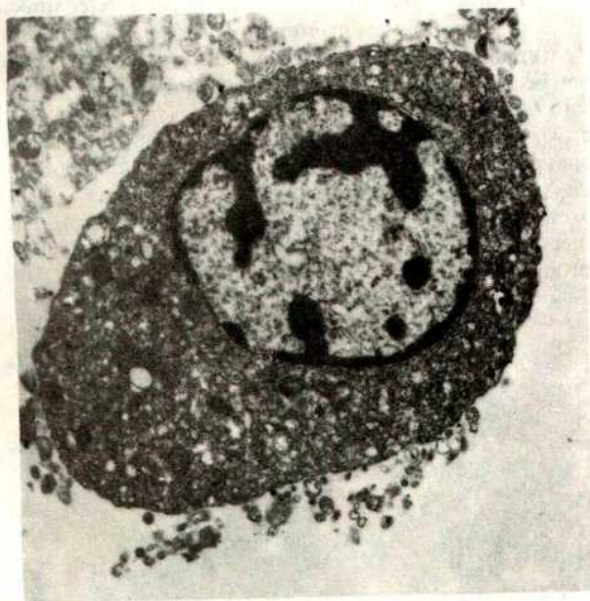


Fig. 2 Limpet haemocyte fixed after 20-s exposure to artificial seawater containing $0.5 \mu\text{g cm}^{-3}$ CB. Note total absence of microspikes. ($\times 12,900$).

microspikes, slowly increase the adhesiveness of their contacts, perhaps by spreading over each other in the same way that they spread slowly on to glass in the presence of CB³; a mechanism which would not be effective in a shaken suspension. The inhibition of rapid aggregation of haemocytes by CB can thus be accounted for entirely by the hypothesis that this fast aggregation depends on the stabilisation of adhesions by rapid spreading of frontal lamellae, in which the CB-sensitive microspikes play an important role (ref. 5 and our unpublished work). It seems possible that other rapidly aggregating cell systems such as blood platelets², thrombocytes¹² and aggre-

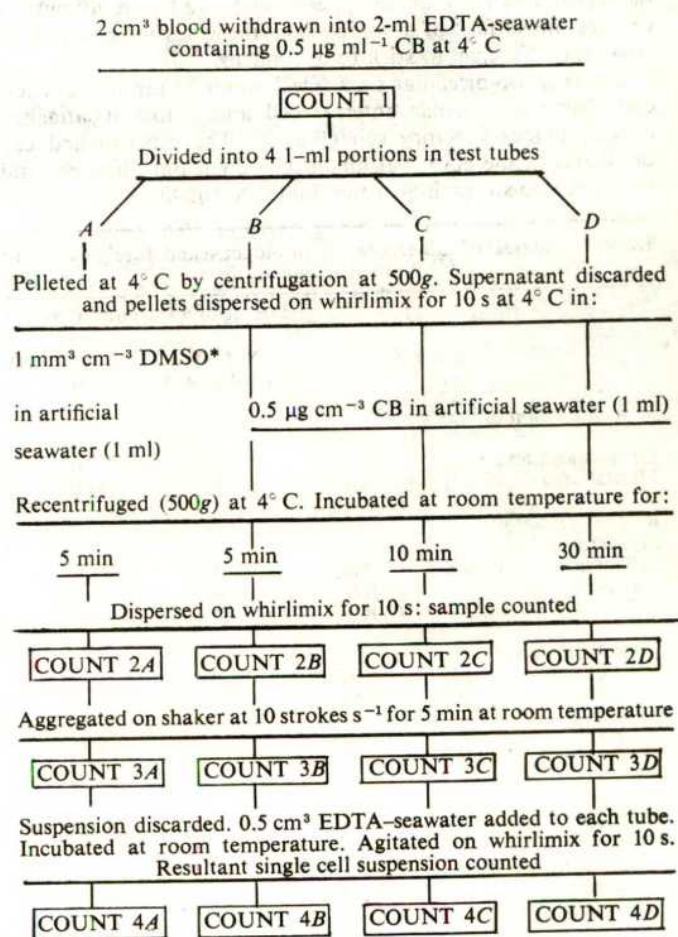


Fig. 3 Diagrammatic representation of experimental procedure used.

*DMSO was used in control experiments because stock CB solution was made up in this solvent.

gating slime mould cells¹³ may operate by a similar mechanism.

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Cell recognition by mucus secreted by urn cell of *Sipunculus nudus*

RECOGNITION of foreign particles in invertebrates is shown by the sticking of these particles to the circulating free amoebocytes of the host. The marine invertebrate *Sipunculus nudus*, phylum Sipunculida, has among its coelomic fluid ('blood') cells a remarkable 'urn cell' which secretes mucus that is sticky for foreign cells, but not for autologous cells. We have studied the biological specificity of this phenomenon and have attempted to alter the surface of autologous erythrocytes to render them sufficiently 'foreign' to stick to urn mucus.

Urn cells are bicellular organelles¹ about 20 µm in diameter, comprising a vesicular anterior cell and a loosely attached ciliated mucus-secreting cell (Fig. 1). The mucociliated cell originates in the epithelial lining of the coelom, detaches and becomes free-swimming in the fluid². Normally, as thousands

of urns swim about in the fluid their small sticky tails remove foreign and cellular debris from the coelom. If pathogenic bacteria or toxic materials are introduced into the coelom, however, urns are stimulated to produce long tails of mucus in which the invading substances are trapped. The host's own 'blood' cells (erythrocytes, non-activated amoebocytes, other white cells), never stick to either normal or actively hypersecreting mucus. Activated amoebocytes stick to both types.

Urn cells can be cultivated *in vitro* in sterile preparations of their own coelomic fluid. One drop of this fluid contains 50-100 swimming urns; their secretory activity can be observed directly in a depression slide by means of a dissecting microscope. The hypersecretory response can be provoked *in vitro* by the stimuli which induce it *in vivo*. Using this *in vitro* system, we had found³ that if the serum of acutely bacteraemic *S. nudus* was filtered and heated to 85°C for 5 min, one drop of this cell-free serum would induce rapid hypersecretion in all urns (Fig. 2). Unheated filtered serum had no effect. The molecular weight of the active molecule in the heated serum was found to be greater than 50,000 (ref. 4).

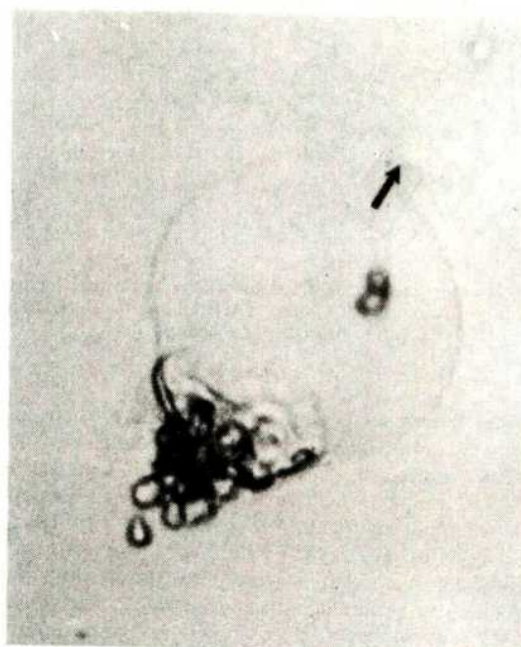
We studied, in the *in vitro* system, the biological specificity of the sticking of foreign cells to urn mucus. When heavy suspensions of *S. nudus* erythrocytes which had been washed three times were added to preparations of either normal or hypersecreting urns, none stuck to the mucus even though urns swam through dense fields of the cells. Table 1 shows that erythrocytes of two geographically isolated (Morgat, Locquêmeau) colonies of *S. nudus*, and from three other species of Sipunculida also failed to stick. Red cells from five unrelated invertebrate species, however, were quickly trapped in both normal and hypersecretory mucus. Human and sheep red blood cells stuck immediately and avidly to normal mucus but much more slowly and less densely to hypersecreted mucus. Sperm from three of the invertebrates were immediately and thickly trapped in hypersecreting mucus; normal secretion has not yet been tested. White blood cells from *Ascidia mentulis*, the crab *Carcinus maenas* and the sea star *Asterias rubens* (the latter two withdrawn into 0.01 M N-ethylmaleimide (NEM) to prevent agglutination) were trapped densely by normal, and somewhat less so by hypersecreting, mucus. Live *Anophrys*, a ciliated protozoan which is tolerated by some invertebrates but is lethal to others, was not trapped; but when inactivated by NEM it was promptly trapped.

Table 1 Degree of adherence of autologous and foreign cells to urn cell mucus

Species	Cell type	Degree of adherence to mucus	
		Normal mucus tails	Hypersecretory tails
<i>S. nudus</i> colonies			
Locquêmeau area	RBC	0	0
Morgat area	RBC	0	0
Related species of Sipunculida			
<i>Phascolosoma vulgare</i>	RBC	0	0
<i>P. elongatum</i>	RBC	0	0
<i>Phascolion strombi</i>	RBC	0	0
Unrelated species			
<i>Arenicola</i>	RBC	+++	+++
<i>Glycera</i>	RBC	+++	+++
<i>Notomastus</i>	RBC	+++	+++
<i>Nereis</i>	RBC	+++	+++
<i>Ascidia</i>	RBC	+++	+++
Sheep	RBC	+++	+
Human	RBC	+++	+
<i>Asterias</i>	Sperm		+++
<i>Ascidia</i>	Sperm		+++
<i>Notomastus</i>	Sperm		+++++
<i>Ascidia</i>	WBC	+++	++
<i>Carcinus</i>	WBC*	+++	++
<i>Asterius</i>	WBC*	+++	++
<i>Anophrys</i> (living)	Protozoan	0	0
<i>Anophrys</i> (inactivated)	Protozoan*		+++

* Withdrawn into 0.01M into N-ethylmaleimide (NEM). Blanks indicate no test.

Fig. 1 Urn cell with normal mucous tail.



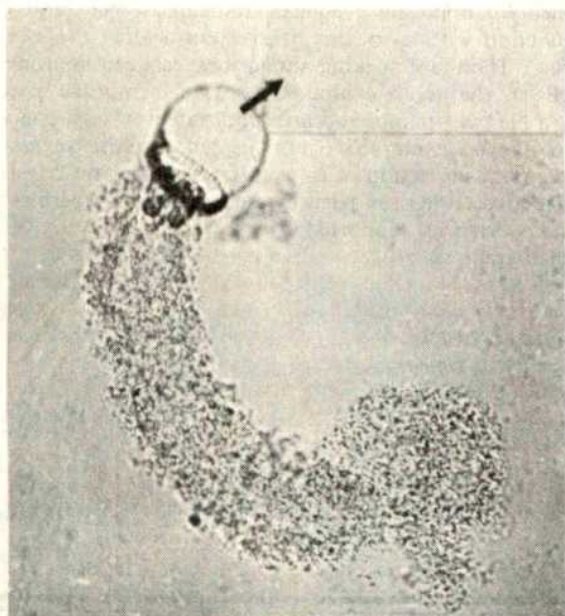


Fig. 2 Hypersecreting urn cell.

We then studied the response of hypersecretory mucus to autologous erythrocytes which were treated in various ways to alter the cell membrane. As Table 2 shows, if red cells were heated to 60° C for 5 min there was moderate sticking, if heated to 85° C for 5 min the heat-killed cells adhered thickly (Fig. 3). If the heat-killed cells were washed in, and resuspended in, a seawater suspension of 1 mM EDTA, they failed to stick. Red blood cells were incubated with five enzymes known to contain powerful proteases and/or glycosidases; with a marine vibrio presumed to contain sialidase (many strains do); and with concanavalin A with the thought that it might bind with cell surface sugars⁵ and mask these sites. Treatment with clarase, which contains α amylase, resulted in sticking of some of the erythrocytes to the mucus, but none of the tested enzymes was as effective as heat-killing.

Roseman⁶ has proposed that cell adhesion may involve an enzyme-substrate interaction between glycosyl transferases and complex carbohydrates. Cell-free urn mucus offers a natural substrate for studies of cell adhesion. Urn cell mucus can apparently recognise (trap) foreign cells from both vertebrate and invertebrate hosts other than sipunculids; whether the mucus or the cell is the determining substrate is an open question. The fact that human and sheep cells are trapped with

differential avidity by normal and by hypersecreted mucus suggests structural differences in the two types of mucus.

It should now be possible to determine (1) what specific chemicals will interfere with the recognition of foreign cells by mucus, and (2) which enzymes will change the host cell surface so that it is recognised as foreign by mucus.

We thank Dr J. Bergerard of the University of Paris, Director of the Station Biologique de Roscoff, and the administrative staff of the station for all facilities; Dr J. Vasserot for inverte-

Table 2 Treatment of *S. nudus* red blood cells in attempts to alter cell membrane and achieve 'foreignness,' as measured by sticking of RBC to hypersecreted urn mucus

Substance used	Time of incubation with RBC	Temperature (°C)	Degree of sticking to mucus
<i>S. nudus</i> RBC	(Unheated)	20	0
<i>S. nudus</i> RBC	Heated 5 min	60	++
<i>S. nudus</i> RBC	Heated 5 min	85	+++
Marine vibrio	30 min	20	0
Rhozyme 1%	30 min	20	0
Bromelain 1%	30 min	30	+
Clarase 1%	30 min	20	++
Hyaluronidase 1%	30 min	20	0
Pronase 1%	30 min	20	+
Concanavalin A 1%	30 min	20	0

Rhozyme HP-150 (Rohm and Haas), from *Aspergillus niger*, contains many glycosidases plus some proteases. Pineapple bromelain (Dole, Hawaii) is a powerful proteolytic enzyme, but also contains glycosidases. Clarase (Miles), from *Aspergillus oryzae*, contains α -amylase and many other glycosidases. ••

brate species other than *S. nudus*, and Dr Y. C. Lee of the Department of Biology, Johns Hopkins University, for advice and counsel, as well as for the test enzymes.

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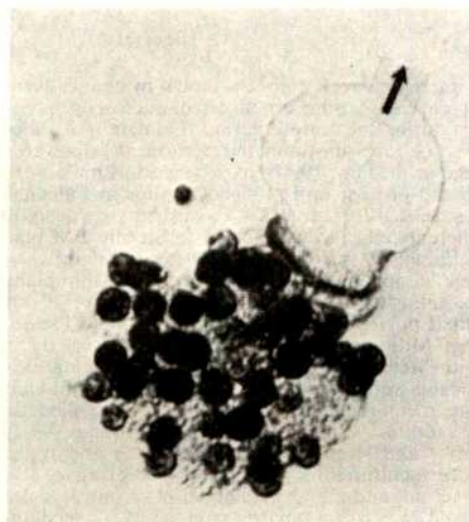
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Fig. 3 Hypersecreting urn cell with adherent heat-killed erythrocytes.



Glucose-induced decrease in Rb⁺ permeability in pancreatic β cells

THE membrane potential of pancreatic islet cells varies with the extracellular D-glucose concentration¹, from about -33 mV at zero glucose to about -16 mV at 28 mM, and 3-17 mM D-glucose induces bursts of rhythmic depolarisations with an amplitude of a few mV. Similar observations have been obtained², but with a membrane potential of about -60 mV in the absence of glucose. Comparisons of various sugars revealed a qualitative correlation between depolarising and insulin-releasing capacities¹; continuous recordings from single islet cells revealed a quantitative correlation between spike frequency and D-glucose concentration^{2,3}. Depolarisation of the β -cell membrane may therefore be essential for the triggering of insulin secretion¹⁻³. We report some experiments which indicate that the diminishing effect of D-glucose on membrane potential may be caused by a decrease in K⁺ permeability.

The ionic basis of membrane potential and rhythmic depolarisations in the pancreatic β cell is largely unknown,

although there are indications that the latter may predominantly reflect Ca^{2+} influx⁴. Using $^{86}\text{Rb}^+$ as a functional analogue of K^+ , we suggested⁵ that a Na^+/K^+ -pump in the β cell is capable of concentrating Rb^+ more than fortyfold from 70 μM RbCl in the extracellular fluid⁵. Measurements of $^{22}\text{Na}^+$ uptake indicated that β cells in equilibrium with a physiological buffer contain as much as 95 mM Na^+ inside⁶. Thus, the relatively small membrane potential in the β cell may tentatively be attributed to a comparatively great permeability to Na^+ , a low Na^+/K^+ -pump activity, or both. Since D-glucose was not found to alter the fluxes of Na^+ (refs 5 and 6), however, the glucose-induced decrease in membrane potential is not readily explained in terms of a further increase in Na^+ permeability or decrease in pump activity.

Fresh pancreatic islets (containing more than 90% β cells), were microdissected free-hand from adult non-inbred *ob/ob* mice and incubated for 2 h at 37°C in Krebs-Ringer bicarbonate buffer equilibrated with $\text{O}_2:\text{CO}_2$ (95:5) and containing (mM): 143.5 Na^+ , 5.9 K^+ , 2.6 Ca^{2+} , 1.2 Mg^{2+} , 128.3 Cl^- , 25.0 HCO_3^- , 1.2 SO_4^{2-} , 1.2 H_2PO_4^- , 3.0 D-glucose, 0.07 $^{86}\text{Rb}^+$ (290 Ci mol⁻¹) and 0.1 6,6'-³H-sucrose (150 Ci mol⁻¹). The same type of medium without Rb^+ and sucrose was used as basal medium in the following. After brief rinsing (~2 s) in basal medium, incubation was continued in basal medium only, or in basal medium supplemented with 17 mM D-glucose, L-glucose or 3-*o*-methyl-D-glucose. The islets were then freeze-dried overnight (-40°C, 0.1 Pa), weighed on a quartz-fibre balance and analysed for radioactivity in a liquid-scintillation spectrometer. Because sucrose is distributed as an extracellular space marker in microdissected islets⁷, the Rb^+ content was corrected for ^{86}Rb in the sucrose space; however, this correction was a minor one, as the sucrose space contained less than 5% of the total Rb^+ content.

Table 1 Content of $^{86}\text{Rb}^+$ in islets preloaded with the isotope and subsequently incubated for 10 min in non-radioactive media of different sugar composition

Sugar in medium	No. of experiments	$^{86}\text{Rb}^+$ (mmol) retained per kg dry weight of islets
D-glucose (3 mM)	18	1.53 ± 0.15
D-glucose (20 mM)	18	1.96 ± 0.10
D-glucose (3 mM) plus L-glucose (17 mM)	17	1.53 ± 0.09
D-glucose (3 mM) plus 3- <i>o</i> -methyl-D-glucose (17 mM)	18	1.57 ± 0.07

Mean values \pm s.e. Control islets (3 mM D-glucose) released about 40% of their Rb^+ content in 10 min; to express results in mM, the tabulated values should be divided by 1.2, as the intracellular water of incubated islets amounts to about 1.2 l per kg dry weight (refs 5 and 7).

Table 1 shows the amount of Rb^+ retained by the islets during a subsequent 10 min incubation in non-radioactive medium. The islets retained significantly more Rb^+ when the extracellular D-glucose concentration was 20 mM than when it was 3 mM ($P < 0.025$). This effect of D-glucose could not be reproduced using L-glucose or 3-*o*-methyl-D-glucose. Since the disappearance of Rb^+ from preloaded islets seems to be an exponential process that probably reflects unidirectional efflux of the ion⁵, the inhibitory action of D-glucose is most simply interpreted as a decrease in Rb^+ permeability. Moreover, as Rb^+ can effectively substitute for K^+ in the Na^+/K^+ -pump and D-glucose had no demonstrable effect on Na^+ fluxes^{5,6}, it seems reasonable to assume that our Rb^+ data reflect properties of the system governing passive K^+ fluxes in the β cell.

The clear specificity of the inhibitory effect of D-glucose is interesting in view of the fact that, among the sugars tested here, only D-glucose is capable of stimulating insulin secretion and of inducing electrical activity in the islet cells¹. According to the generally accepted theory of the genesis of neuro-membrane potential, the decisive factors are an active estab-

lishment of opposing chemical gradients of Na^+ and K^+ in conjunction with a greater passive permeability to K^+ than to Na^+ . There are striking similarities between neurones and β cells in that both exhibit a negative membrane potential, have a Na^+/K^+ -pump, and are depolarised by high concentrations of extracellular K^+ (ref. 4). We therefore suggest that the depolarising action of D-glucose on pancreatic β cells may be mediated, at least in part, by a decrease in K^+ permeability.

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Macrophage proliferation *in vitro* induced by exudates

MOUSE peritoneal macrophages in culture are blocked in the G_0 phase of the cell cycle¹ and can be stimulated to synthesise DNA and divide *in vitro* only by exposure to feeder layers or to medium conditioned by other cell types²⁻⁴. A similar system has been reported for mouse alveolar macrophages⁵. This reluctance to divide *in vitro* contrasts with the striking proliferation of inflammatory macrophages *in vivo*^{6,7}.

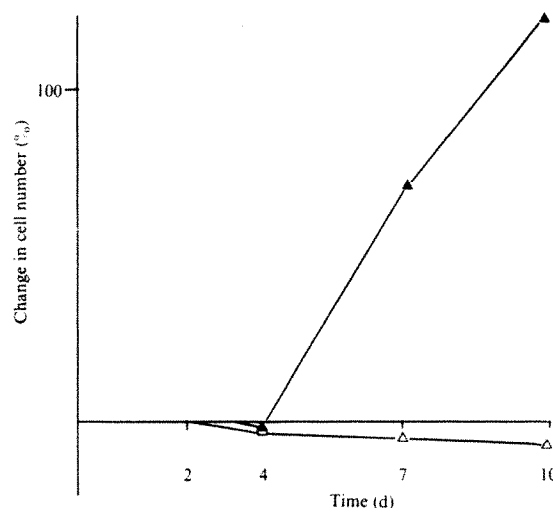


Fig. 1 Increase in cell numbers shown by counts over the same area of coverslip using an inverted microscope at sequential intervals during the culture period. Exudate was added at time zero. Cell cultures unstimulated peritoneal cells were obtained by lavage in medium 199 from young adult male Porton mice, were seeded on to 6 mm glass cover slips in Falcon Microtest II plates; settled for 2 h at 37°C, washed vigorously to remove non-adherent cells and transferred to Sterilin LM plates sealed with a 19 mm glass coverslip. Cell-free exudate was raised in the same donor mice by the subcutaneous implantation of modified diffusion chambers. These consisted of 20 mm lengths of nontoxic polystyrene tubing (10 mm diameter) sealed at each end by a Millipore membrane filter (pore size 0.45 μm) and filled with Medium 199. Four days after implantation, protein-rich exudates were recovered from the chambers, filtered through Millipore membrane filters (0.45 μm) and applied directly to the cell cultures. Culture media were medium 199 (penicillin 100 U ml⁻¹ and streptomycin 100 μg ml⁻¹) and foetal bovine serum. Test cultures (▲) received 50% exudate, 25% foetal bovine serum and 25% medium 199. Control cultures (△) received 75% foetal bovine serum and 25% medium 199.

We have now been able to initiate *in vitro* proliferation in cultures of mouse peritoneal macrophages by the addition of inflammatory exudates. Such cultures exhibited extensive nuclear incorporation of tritiated thymidine, a high incidence of mitotic figures and a considerable increase in total cell numbers as demonstrated by direct counting (see Fig. 1) compared with control cultures not exposed to exudate.

Macrophage proliferation was seldom demonstrable earlier than four days after addition of exudate to the culture. Phagocytosis of human red blood cells and heat-killed *M. tuberculosis*, and histochemically evident acid phosphates, seemed higher in cultures treated with exudate than in the controls. Electron microscopy revealed features typical of macrophages, and the cells treated with exudate seemed to have more cytoplasmic organelles and fimbriae than control cells. Preliminary characterisation of the mitogenic factor present in the exudate has so far indicated only that it is relatively thermostable, being unaffected by freezing and thawing, storage for 6 months at -25°C or by incubation for 1 h at 65°C . It is not yet possible to correct it with the macrophage-derived factors which affect mitosis in other cell types⁸.

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Induction of DNA synthesis in rat macrophages *in vitro* by inflammatory exudate

THE proliferation of macrophages in inflammatory exudates¹⁻³ contrasts with the failure of these cells to divide *in vitro* except in the presence of highly specialised conditioned media⁴⁻⁷. Macrophage division *in vivo*, however, could be explained if inflammation produced a local mitogenic factor.

Inflammatory exudates were produced in DFA-specified pathogen-free rats by intrapleural injection of 1 ml 6% Dextran (40,000 daltons) and collected 4 h later. The exudate was centrifuged and the supernatant passed through a Millipore filter (pore size 0.22 μm). The filtered exudate was diluted with medium 199 plus 20% newborn calf serum to make a final concentration of exudate of 30 or 50%.

Macrophages were collected from DFA rats by washing the peritoneum with medium 199. Macrophages were also collected 4 d after intraperitoneal injection of nutrient broth (activated macrophages). The macrophages were then cultured in Leighton tubes⁸ and 3 d later the medium was replaced with medium containing exudate. Three to six days after the addition of exudate DNA synthesis was assessed by autoradiography after incorporation of a single 30 min pulse of tritiated thymidine. The results of a typical series of experiments (Fig. 1) show that both normal and activated macrophages responded after 5 d of exposure to exudate *in vitro*. The labelling index of the treated cells rose to 20–30% compared with virtually zero in the control macrophages. Samples taken earlier than 5 d showed much less labelling. The macrophages synthesising DNA showed avid phagocytosis of starch particles added to the medium, which seemed to be greater than in the controls. Preliminary experiments have shown that exudates from Lewis rats are active on peritoneal macrophages from inbred Lewis rats and also on mouse peritoneal macrophages.

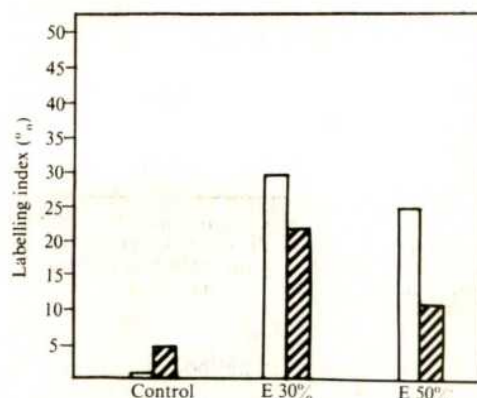


Fig. 1 Uptake of tritiated thymidine by 'normal' and 'activated' macrophages 5 d after culturing with or without exudate added to the culture medium. The open blocks represent normal and the hatched blocks activated macrophages.

The chemical nature of this stimulatory factor is unknown and the possibility of it being a virus cannot be excluded. It does not seem, however, to result from treatment with dextran alone, as dextran added to culture medium and passed through Millipore filters failed to induce DNA synthesis in macrophages. Its relationship to the factors released from peritoneal macrophages which stimulate or inhibit other cell lines remains to be determined⁹.

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Effect of antibody emission rates on plaque morphology

CUNNINGHAM and Fordham¹ have used a modified version of the haemolytic plaque technique to show that antibody diversity can be generated after B cells have been stimulated to proliferate. They tested antibody plaque forming cells (PFC) against a mixture of sheep red blood cells (SRBC) from two different sheep and obtained three morphological classes of direct plaques: clear (the plaque radii for both types of SRBC were equal), sombrero (the plaque radii were different for the two types of SRBC), and partial (there was only one plaque radius). For the clear and sombrero plaques both types of SRBC lysed to some extent, while for the partial plaques only one type of SRBC lysed. Cunningham and Fordham used plaque morphology as an antibody specificity marker, that is, if two plaques were different in morphology this was taken to mean that the antibodies produced by the two PFC differed in their specificity for the two types of SRBC. They studied clones produced from single PFC and found in most cases that the plaques produced by the progeny were of the same morphological class, differing only by slight variations in their plaque radii. In 10 of the 93 clones observed, however, the morphology of the plaques differed within the clone. In 7 clones two different morphological classes of plaque were observed, and in the others all three classes. Cunningham and Fordham suggested that within a given clone the antibodies emitted by different cells can have different specificities.

We report here that changes in plaque morphology do not

Table 1 Plaque radii

S (antibodies s^{-1})	r_1 (mm)	r_2 (mm)	A_1/A_2
10^2	0.59	0.02	870
10^3	0.76	0.15	36
10^4	0.94	0.28	11
10^5	1.10	0.45	6

S , antibody (Ab) emission rate; r_1 , distance from the PFC to the edge of lysis of type 1 SRBC; r_2 , distance to the edge of lysis of type 2 SRBC. The last column lists the ratio of the areas of lysis. These radii were calculated using equations (5) and (6) and the values of the parameters listed below Fig. 1.

necessarily indicate changes in antibody specificity, but can be caused by changes in the rate at which antibodies are emitted from the PFC, that is, two plaques formed by antibodies with identical specificities can be of different morphological classes if the antibody emission rates of the PFC differ.

If we consider lymphocytes which emit antibodies with identical specificities that form multivalent attachments to epitopes on the surface of type 1 SRBC and monovalent attachments to epitopes on the surface of type 2 SRBC, then at low antibody emission rates the concentration of bound antibodies on type 2 SRBC may be too low to produce observable lysis in the presence of complement so that a partial plaque is obtained. At high antibody emission rates the bound antibody concentration will be higher on both types of SRBC. If the concentration is high enough on the type 2 SRBC a sombrero will occur. I show here that such changes in plaque morphology can occur for reasonable values of the binding parameters and emission rates.

Mathematical descriptions of plaque formation due to single antibody emitting cells in thick gels containing SRBC have been presented^{2,3}. It is straightforward to modify the theory for a thin layer (diffusion only in a plane) containing two types of SRBC. For the case just discussed, in which the antibodies form multisite attachments to binding sites on type 1 SRBC (strong binding) and single site attachments to binding sites on type 2 SRBC (weak binding), equations (1)–(3) describe such binding where there is a lymphocyte at the origin emitting antibodies (IgM in our case) at a rate S , and where these antibodies diffuse into a layer containing a homogeneous mixture of the two types of SRBC.

$$\frac{\partial c}{\partial t} = D \nabla^2 c + \frac{1}{n} \frac{\partial \rho_1}{\partial t} + \frac{\partial \rho_2}{\partial t} + \frac{S}{h} \delta(r) \quad (1)$$

$$\frac{\partial \rho_1}{\partial t} = -k_{11} n c \rho_1 \quad (2)$$

$$\frac{\partial \rho_2}{\partial t} = -k_{21} c \rho_2 + k_{22} (\rho_{20} - \rho_2) \quad (3)$$

c is the concentration of free antibody, ρ_1 and ρ_2 the concentration of free binding sites on type 1 and 2 SRBC, h the layer thickness, k_{11} the antibody–SRBC1 forward rate constant, n the average number of bound sites per multisite attachment, k_{21} and k_{22} the antibody–SRBC2 forward and reverse rate constants, r the position vector (two-dimensional), $\delta(r)$ the two-dimensional Dirac delta function, D the diffusion coefficient of the antibody and ∇c the concentration gradient of antibody. At time $t = 0$, $c = 0$, $\rho_1 = \rho_{10}$ and $\rho_2 = \rho_{20}$. In equation (2) the change in the binding site concentration caused by detachment of antibodies bound multivalently is neglected. This is equivalent to assuming that once an antibody binds multivalently it remains bound until the end of the experiment (one hour or less in this case).

When the fraction of binding sites occupied is small these equations can be linearised and solved^{2,4}. (Note that the fraction bound can be small even though large numbers of antibodies are bound per RBC. This is because of the large number of sites per RBC, for example, 6×10^5 sites per SRBC for the Forssman epitope⁵.) For times longer than a few seconds local equilibrium will exist between the free and monovalently bound antibodies.

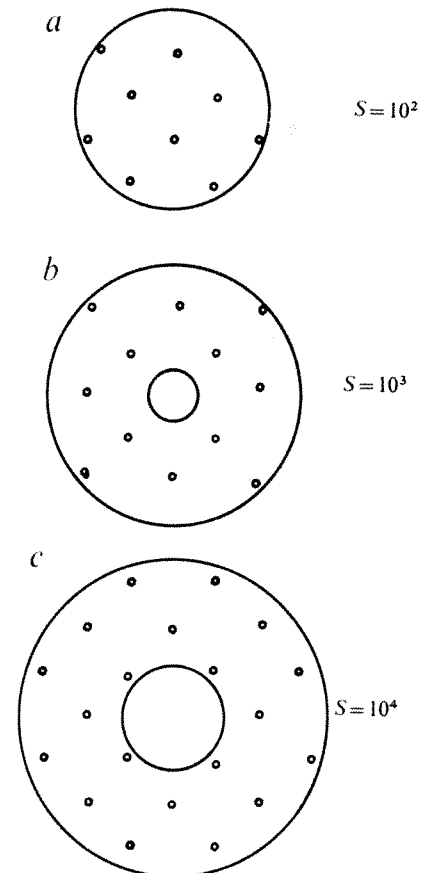


Fig. 1 Three plaques calculated from equations (5) and (6) for three values of the emission rate S . The values of the plaque radii are listed in Table 1. The clear area represents lysis of both type 1 and 2 SRBC. The area with small circles present represents lysis of only type 1 SRBC. Beyond the outer circle there is no lysis. Plaques with radii smaller than 0.05 mm are assumed to be non-observable. *a*, $S = 10^2$ antibodies s^{-1} . The plaque is partial. *b*, $S = 10^3$ antibodies s^{-1} . *c*, $S = 10^4$ antibodies s^{-1} . The plaques are sombrero. The values of the parameters used to calculate the plaque radii in Table 1 are as follows: $E_1 = 10^3$ sites per RBC, $E_2 = 5 \times 10^3$ sites per RBC, $K = 10^6 M^{-1} = 1.66 \times 10^{-15} cm^3$ per antibody, $n k_{11} = 10^3 M^{-1} s^{-1} = 1.66 \times 10^{-16} cm^3$ per antibody s^{-1} , $h = 2 \times 10^{-3} cm$, $D = 10^{-7} cm^2 s^{-1}$, $\rho = E_1 \rho_{0RBC}$ and $\rho_{RBC} = 2 \times 10^8 RBC cm^{-3}$, $n = 2$ and $t = 3,600 s$. The number of IgM molecules bound per SRBC at the plaque radius was taken to equal one. For a discussion of these parameters see ref. 3.

Further, for the values of the parameters we consider, $k_{11} \rho_{10} t \gg 1$, in which case the solutions to equations (1)–(3) become:

$$c(r, t) = (S/2\pi D h) K_0(\lambda r) \quad (4)$$

$$N_{b1} = E_1 \{1 - \exp[-k_{11} n t c(r, t)]\} \quad (5)$$

$$N_{b2} = K E_2 c(r, t) \quad (6)$$

(In general $c(r, t)$ can be written as the sum of a time-independent term, equation (4), and a time-dependent term. When $k_{11} \rho_{10} t \gg 1$, the time-dependent term is negligible.) N_{b1} and N_{b2} are the number of bound antibodies per SRBC at position r and time t on type 1 and type 2 SRBC, E_1 and E_2 are the number of binding sites on type 1 and 2 SRBC at $t = 0$, $K = k_{21}/k_{22}$, K_0 is a modified Bessel function⁶ and $\lambda = (k_{11} \rho_{10} / D)^{1/2}$.

To obtain values for the plaque radii we must know the number of antibodies bound per SRBC at the edge of the plaque. For IgM this number has been estimated to be between one and ten³. We take it to equal one. When we set $N_{b1} = N_{b2} = 1$, equations (5) and (6) become equations for the plaque radii r_1 and r_2 , respectively. Using these equations we can predict how the plaque radii change as functions of the various parameters.

Figure 1 and Table 1 show how the plaque size and morphology vary as a function of antibody emission rate. The three plaques in Fig. 1 were obtained for $S = 10^2, 10^3$ and 10^4 anti-

bodies s^{-1} . (Experimental studies have yielded estimates of S in the range 0.5×10^{-1} – 2×10^4 antibodies s^{-1} (ref. 7).) All the parameters except S have been held fixed, including the various binding constants. Thus the antibody specificity is the same for the three plaques in Fig. 1. By increasing S the plaques change from partial to sombrero.

We have shown that differences in the antibody emission rate of a clone member can cause it to produce a plaque which differs in morphology from those plaques produced by other clone members. We have demonstrated transitions between partial and sombrero plaques. One other question to consider is whether changes in the antibody emission rate can cause progeny of a clear PFC to yield other than a clear plaque. If the antibody binding constants are identical for types 1 and 2 SRBC, a clear plaque will be produced for all antibody emission rates. A clear plaque, however, does not necessarily mean the antibody has identical binding constants for both types of SRBC. It may instead mean that the differences in specificity are not sufficient to produce a measurable difference in the plaque radii. Whether a large change in the emission rate could lead to two well defined plaque radii (a sombrero) is an open question.

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DR CUNNINGHAM REPLIES—Plaque morphology has been used^{1–5} as a simple way of distinguishing cells making different antibody specificities. Other interpretations of the meaning of plaque differences are possible, and Goldstein⁶ has suggested that rate of antibody secretion is decisive. As with any complex assay system, it is impossible to discount such alternative interpretations absolutely. The question is not, 'are such artefacts conceivable?'—for evidently they are—but 'are they likely to occur under the experimental conditions?' Control experiments and arguments for the validity of plaque morphology as a specificity marker have been presented elsewhere⁷. Some of these are listed below.

Goldstein's argument requires that plaques with different morphologies should have different overall (largest) diameters. In fact, in many of our clones, the outer diameter of partial lysis was similar in most or all plaques, while the clear area of total lysis varied from one cell to the next. In effect, overall plaque size acts as a control against differences in rate of antibody production per unit time. His argument could, however, be rescued with more complicated assumptions, for example rapid fluctuations in rate of antibody release.

To eliminate this we watched plaques develop. Those which started as sombreros (after incubation for 15 min) always grew into larger sombreros with the same relative areas of clear and partial lysis at 1 h (ref. 7). Clear and partial plaques, (the 'extreme cases' of sombrero morphology), behaved similarly.

Individual plaque-forming cells, micromanipulated from one red cell monolayer to another, produced plaques of similar morphology at different temperatures⁷. That is, rate of antibody release did not seem to affect plaque morphology.

Clones grown *in vivo*^{1,8} often contained several thousand antibody-forming cells all with exactly the same morphology on a mixed indicator monolayer. Others had varying proportions of two types, for example half were clear and half partial, with no intermediate forms. It is not an indisputable argument, but if plaque morphology depends critically on rate of antibody

release, one would expect to find a spectrum of types in any collection of plaques. This observation of very large numbers of homogeneous plaques also serves to discount the suggestion that variation in rate of production is a rare event which our control experiments failed to detect.

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Temperature-sensitive growth of cells transformed by *ts-a* mutant of polyoma virus

ONE of the characteristics of the transformed state in culture in mammalian fibroblasts transformed by polyoma virus and Simian Virus 40 (SV40) is the lowered serum requirement for cellular growth^{1–4}. The participation of a viral gene(s) in this attribute of transformation has been known for polyoma virus⁵ and SV40 (refs 6 and 7). Recent experiments show that some of the other properties associated with the transformed state are also under the control of the same viral gene(s)^{8–11}. The *ts-a* gene of polyoma virus controls the initiation of stable transformation in the hamster BHK 21 cell line¹², although it is usually accepted that the function of this gene is not required for the maintenance of the transformed state^{12–14}. I report here the temperature-sensitive growth in low serum medium of some of the rat cell lines transformed by the *ts-a* mutant of polyoma virus, and suggest that this viral gene controls at least one aspect of the maintenance of transformed state in certain transformed cells.

Like other untransformed fibroblastic cells widely used in transformation experiments^{1–3,15}, 3Y1 rat cells require a high concentration of serum for growth and exhibit poor growth in medium containing low concentrations of serum, whereas 3Y1 cells transformed by polyoma virus or SV40 can grow well in low serum medium (Kimura and Kaneto, unpublished). To determine whether the *ts-a* gene of polyoma virus affects the growth properties of transformed cells, seven independent 3Y1 lines transformed by *ts-a* mutant at permissive temperature as well as three 3Y1 lines transformed by wild type (WT) virus were examined for their ability to grow in low (2%) and high (10%) serum medium at 33°C and 40°C, the permissive and non-permissive temperatures for the productive cycle of this mutant^{15,19}. Figure 1 shows that the growth of untransformed 3Y1 cells was reduced in 2% serum at both 33°C and 40°C, compared with that in 10% serum. Growth in 2% serum of three of the 3 WT-transformed lines (Py-3Y1-3, 4 and 117) was also reduced at both temperatures, compared with that in 10% serum, but it was still much better than that of 3Y1 in 2% serum at both temperatures. A striking difference in cellular growth was observed in 3Y1 lines transformed by *ts-a* mutant. There was much less growth in four out of the seven *ts-a*-3Y1 lines (*ts-a*-3Y1-1, 2, 3 and 6) in 2% serum at 40°C than at 33°C. Two lines (*ts-a*-3Y1-3 and 6) further showed less growth in 10% serum at 40°C than 33°C. Three *ts-a*-3Y1 lines (*ts-a*-3Y1-4, 5 and 7) showed little or no detectable temperature-sensitivity for growth under the conditions, thus resembling WT-transformed 3Y1 cells. Figure 2 shows the growth curves of one of the *ts-a*-transformed 3Y1 lines (*ts-a*-

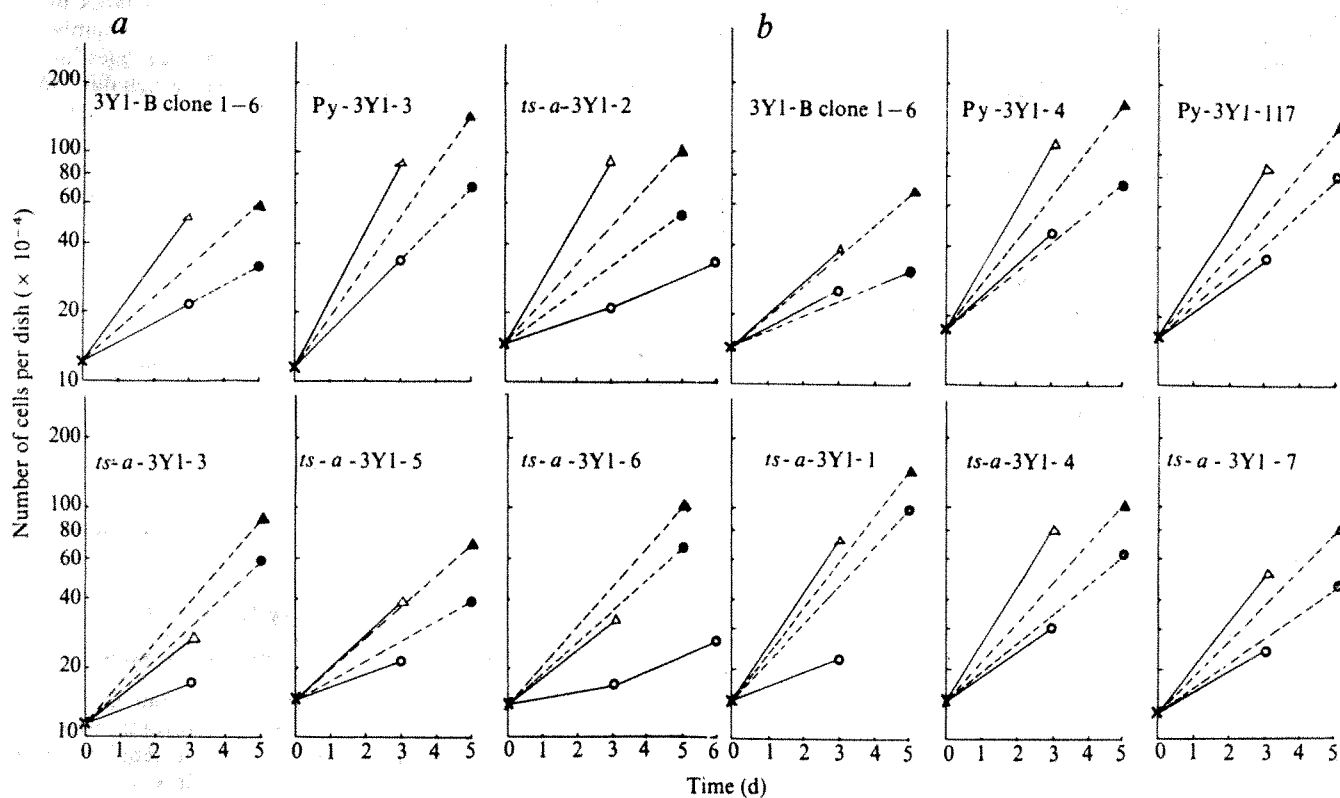


Fig. 1a and b. Growth in different serum concentrations of 3Y1 cells at 40°C and 33°C after transformation by polyoma virus and by *ts-a* mutant of polyoma virus at permissive temperature. Cells grown at 33°C (transformed lines) and at 37°C (3Y1-B 1-6) in medium containing 10% foetal bovine serum were dispersed with a trypsin-Versene mixture, suspended in Vogt-Dulbecco medium containing 1.5% serum, counted in a haemocytometer and diluted in the same medium. The cell suspension (5 ml) was inoculated into each 50-mm plastic Petri dish (2×10^5 cells per dish). After incubation at 33°C for 3 h to allow cells to settle, the cultures were replaced with 5 ml of fresh medium containing 2% or 10% serum. Half of the cultures were placed in an incubator at 40°C, the other half at 33°C (zero time). At zero time and at indicated times of incubation, cells were trypsinised and counted. Accuracy of cell count is within 10%. *a* and *b* represent different experiments: ○, 2% serum at 40°C; △, 10% serum at 40°C; ●, 2% serum at 33°C; ▲, 10% serum at 33°C. A stock of WT polyoma virus (M. Vogt, clone LP147) was prepared in mouse kidney cultures. A stock of *ts-a* mutant of polyoma virus¹⁵ (Dulbecco) was prepared in BALB/3T3 cells. All transformed lines used were derived from a cloned population of the rat cell line 3Y1 (3Y1-B clone 1) (ref. 16, and G.K., A. Itagaki and J. Summers, unpublished). The 3Y1 cells were infected with WT or *ts-a* at an input multiplicity of about 50 and incubated either at 37°C (WT) or first at 33°C for 2 d and then at 37°C (*ts-a*). After 2–3 weeks, transformed colonies were identifiable as the relatively thick colonies composed of cells randomly oriented and piled up on each other (criss-cross) as described previously (G.K., A. Itagaki, and J. Summers, unpublished). Transformed cell lines were developed at 37°C by choosing well isolated colonies. Only one colony was selected from the given Petri dish, to obtain independent cell lines. To avoid possible secondary alterations, care was taken to prevent crowding. The cell lines were stored at –70°C at their early passages. The transformed cells were grown and passaged at 33–37°C for 9–12 d after thawing and grown at 33°C for 2 d immediately before assay for cell growth. Untransformed 3Y1 cells were grown at 37°C until assay. Cells were cultivated in plastic Petri dishes (Falcon) in Vogt and Dulbecco's modification of Eagle's MEM (ref. 17) with a glucose concentration of 1 g l^{-1} supplemented with, unless otherwise specified, 10% foetal bovine serum in a humidified incubator flushed with a CO_2 -air mixture.

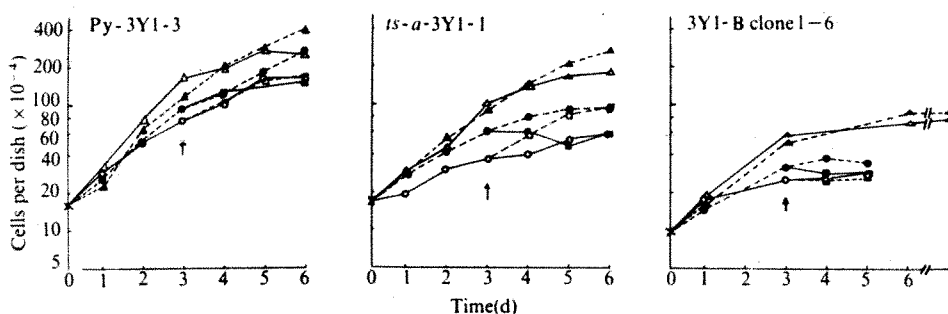


Fig. 2 Growth curves at 40°C and 33°C at different serum concentration and effect of temperature-shifts on the growth of 3Y1, polyoma-transformed 3Y1 and *ts-a*-transformed 3Y1 cell lines. Cells grown at 33°C (transformed lines) and 37°C (3Y1-B 1-6) were dispersed with trypsin-Versene, pelleted at 1,500 r.p.m. for 5 min, and suspended in Vogt-Dulbecco medium containing 1.5% foetal bovine serum and 10 units ml^{-1} of mycostatin (Squibb). Aliquots of the cell suspension (5 ml, 2×10^5 cells) were inoculated into each 50-mm plastic Petri dish. After incubation at 33°C for 3–6 h, medium was replaced with 5 ml of the same medium containing indicated amounts of foetal bovine serum and the experimental incubation started at 40°C and 33°C (zero time). On day 3 (arrow) some cultures were shifted from 40°C to 33°C and some from 33°C to 40°C. Medium was not changed throughout the incubation. At indicated times cells were trypsinised and counted. At least 200 cells were counted for each point. ○, 2% serum at 40°C; △, 10% serum at 40°C; ●, 2% serum at 33°C; ▲, 10% serum at 33°C; □, 2% serum from 40°C to 33°C; ■, 2% serum from 33°C to 40°C.

Table 1 Virus production by rat 3Y1 cells transformed by *ts-a* mutant of polyoma virus on cell fusion with BALB/3T3 cells*

Cell line	Virus titre (plaque forming units per 10 ⁶ transformed cells)		
	Plus none (0 time)†	Plus BALB/3T3	Plus BALB/3T3 and Sendai
<i>ts-a</i> -3Y1-1	<5	<5	<2.5×10 ¹
<i>ts-a</i> -3Y1-2	0	<2.5×10 ¹	4.7×10 ³
<i>ts-a</i> -3Y1-3	0	1.6×10 ³	2.2×10 ⁴
<i>ts-a</i> -3Y1-4	0	2.5×10 ¹	1.0×10 ⁴
<i>ts-a</i> -3Y1-5	0	2.0×10 ²	<2.5×10 ¹
<i>ts-a</i> -3Y1-6	0	<2.5×10 ¹	1.5×10 ⁴
<i>ts-a</i> -3Y1-7	ND	ND	ND

*Transformed cells (10⁶) to be fused were mixed with 10⁶ BALB/3T3 cells in 0.5 ml Vogt-Dulbecco medium. The mixture was added to 0.5 ml of the allantoic fluid containing 4,000 haemagglutinating units of ultraviolet-inactivated Sendai virus (HVJ-Z strain, Y. Okada). After occasional gentle shakings at 4–10°C for 10 min, and subsequent incubation at 37°C for 30 min without shaking, the mixture was poured into a 50-mm Petri dish with 4 ml of medium. Incubation was carried out at 33°C for 6 d with a medium change on day 1. Cells were broken open into the medium by freeze-thawing and virus was titred by plaque formation on BALB/3T3 monolayer at 33°C and 39°C.

†Transformed cells (10⁶) suspended in 2.5 ml Eagle's MEM were broken open by freeze-thawing more than five times and were assayed for virus by plaque formation at 33°C using ten Petri dishes for each transformed line.

ND, not determined.

3Y1-1) as well as those of a WT-transformed 3Y1 line (Py-3Y1-3) and of untransformed 3Y1. Growth of *ts-a*-3Y1-1 cells was again very slow in 2% serum at 40°C. When cells grown in 2% serum were shifted from 40°C to 33°C and from 33°C to 40°C, resumption and slowing down, respectively, of the growth of *ts-a*-3Y1-1 cells occurred within a 24 h period, suggesting that the temperature-sensitive reaction is reversible.

As we used a cloned population of 3Y1 cells, from which all the transformed lines studied were derived, it is unlikely that WT and *ts-a* viruses are transforming different variants in the same cell population. It has already been shown that ten out of eleven 3Y1 cell lines independently transformed by WT polyoma do not produce virus spontaneously, at least during their early passages, and are free of infectious polyoma (G. K., A. Itagaki and J. Summers, unpublished). Eight of the ten virus-free transformed lines including the three lines used in this study produce virus on fusion with permissive mouse cells (G. K., A. Itagaki and J. Summers, unpublished). The *ts-a*-3Y1 lines were tested for their ability to produce virus on fusion with BALB/3T3 cells. Table 1 shows that infectious virus was not detected in extracts of all six of *ts-a*-3Y1 lines using the direct plaque assay. When cocultivated with BALB/3T3 cells, three *ts-a*-3Y1 lines produced a small amount of virus. When the cocultivation was carried out in the presence of Sendai virus inactivated by ultraviolet light, four of the six *ts-a*-3Y1 lines tested produced a relatively large amount of virus, suggesting that heterokaryon formation is necessary for virus production. The virus rescued from each transformed line was identified as the originally transforming *ts-a*, as shown by its temperature-sensitive character for plaque formation and by neutralisation with polyoma virus antiserum. These results, together with the morphological characteristics of transformed colonies described above, suggest that the transformed lines studied here were actually transformed by the virus strains under study.

I suggest that the *ts-a* gene of polyoma virus controls at least one aspect of the maintenance of transformed state in certain transformed cells, that is, the ability of transformed cells to grow in low serum medium. Whether or not the *ts-a* gene determines other properties associated with the transformed state remains to be seen.

Why three of the seven *ts-a*-3Y1 lines showed the WT phenotype is not clear. It is unlikely that these lines were transformed by the revertant virus, since the virus rescued from one of the lines (*ts-a*-3Y1-4) was found to be still tem-

perature-sensitive. A possible explanation is that secondary cellular alterations occurred during the passages of cells after the initial transformation event. Second, the virus may have been transformed, by chance, pre-existing variants which might even have arisen by way of the small number of passages of the parental clone of 3Y1 cells. Third, the *ts-a*-3Y1 cells which are not temperature-sensitive might contain more viral gene copies than do the temperature-sensitive cells, so that they would have more gene product. Where there is a lot of product the cell line might function partially at 40°C, so that these cells would not be temperature-sensitive. It might also be possible that the *ts-a* gene product could interact with more than one factor or receptor site in transformed cells. The nature of the mutational change in the gene product and the nature of the cellular factor would both determine the success of the interaction.

The *ts-a* function is known to be required for initiation of each new round of polyoma viral DNA replication in productive infection^{19,20}. The same gene function seems to control both the initiation of transformation²¹ and at least one aspect of maintenance of the transformed state. Transformation could be a consequence of the introduction into a cell of the capacity for aberrant initiation of DNA replication. A similar situation has been found and discussed more extensively as a result of studies of the SV40 mutants which have properties similar to those of the polyoma *ts-a* (refs 6, 7, 10 and 21).

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Density and dose-response curve of acetylcholine receptors in frog neuromuscular junction

THE description of the reaction between acetylcholine (ACh) and receptors in the end-plate membrane requires determination of the dose-response curve. The most appropriate response to measure is the conductance change of the voltage clamped end-plate^{1,2}. Recent improvements of the microiontophoretic method^{3,4}, as well as direct visual control of the ACh-pipette in relation to the nerve terminal⁵, enabled us to use the focal application method in a reasonably quantitative way. Only

end-plates with a sole, straight running terminal were chosen for the experiments³⁻⁵, and ACh was always applied at the end of a terminal. The known geometrical arrangement allowed us to calculate the local ACh concentration in amplitude and time course at each point of the terminal by the diffusion law, and to compare it with the measured response of the voltage clamped end-plate. Our results indicate that at least three molecules of ACh react with one receptor unit.

If l is the distance between the site of ACh release and a location x on the terminal, then the local ACh concentration C is given as a function of time t by^{6,7}

$$C = [Q/8(\pi Dt)^{3/2}] \exp(-l^2/4Dt), \quad r^2 = x^2 + z^2 \quad (1)$$

where Q is the number of molecules released at $t = 0$, $l = 0$, D the ACh-diffusion coefficient, z the distance of the ACh-pipette to the end of the terminal. It is assumed that the interaction of ACh with the receptors, at each local site, can be described by the law of mass action (n th-order reaction)^{1,2}

$$y = 1/(1 + (K/C)^n) \quad (2)$$

where y is the fraction of receptors occupied at steady state, K a constant and n the Hill coefficient. If the local conductance change, g , is proportional to the occupancy y , $g = g_{max}y$, then the summed response, g_{sum} , of all the distributed receptors can be described as

$$g_{sum} = \int_0^l g d\lambda = g_{max} \int_0^l \frac{1}{1 + (K/C)^n} d\lambda \quad (3)$$

g_{max} is the maximal conductance per micrometre length of the terminal, l the length of the terminal. This equation takes

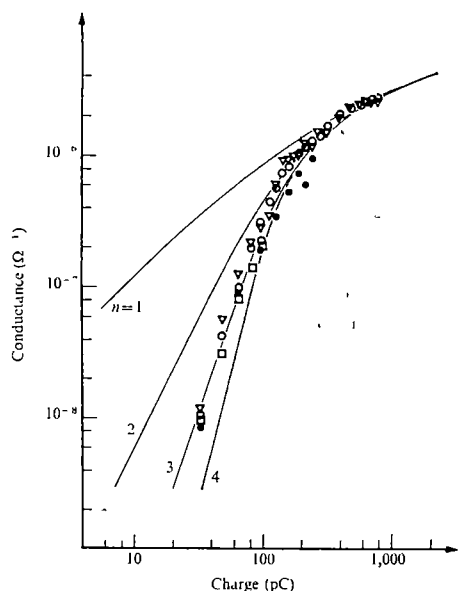


Fig 1 ACh dose-response curve for a voltage clamped frog neuromuscular junction. Symbols, measured peak values of end-plate conductance change, for ACh released 10 μ m above the end of a linear terminal. The ACh pipette was positioned four times (different symbols). Holding and resting potential -85 mV. Two intracellular micropipettes were used for a 'point' voltage clamp. The conductance change of $10^{-6} \Omega^{-1}$ corresponds to 70 nA of end-plate current. Calculated curves according to the equations given in text. Ordinate, conductance change of end-plate, abscissa, charge through iontophoretic pipette. Temperature, 23°C.

into account that with increasing doses more distant receptors contribute to the response. The integral can only be solved numerically. The calculated peak values of g_{sum} were plotted (log-log) against the dose Q . This resulted in standard dose-response curves for a linear terminal, which have a limiting slope of n for low doses, but for high doses show an increase of the response with $Q^{1/3}$ instead of saturation. The shapes of the standard curves are independent of the respective K and g_{max} . Thus, experimental data can be approximated by

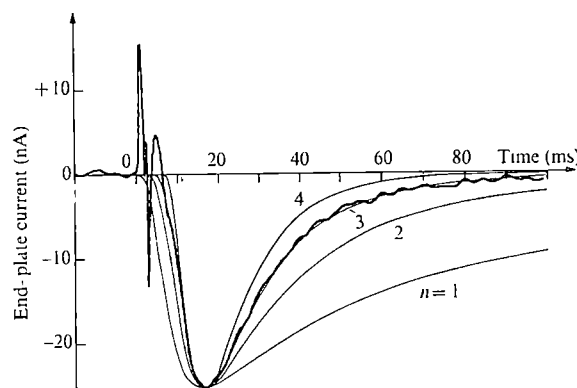


Fig 2 Time course of measured end-plate current (—, pen recorder readout from digitally stored response). One response of those plotted in Fig 1. Charge through ACh-pipette 120 pC (80 nA, 1.5 ms). Record starts with an artefact from the ACh-pulse. —, Theoretical responses $g_{sum}(t)$ according to the equations given in text. The curves were plotted by coinciding peak time and peak amplitude.

shifting the standard curve until the best fit is obtained, and g_{max} and K (concentration for 50% activation of g_{max}) can be determined.

The result of an experiment where ACh was released 10 μ m above the end of a linear terminal of a voltage clamped end-plate is illustrated in Fig 1. The limiting slope at low doses (for determination of n) and the bend into the saturation slope (for determination of g_{max}) are easily seen on the curve. The experimental data are well described by equation 3 (solid line), assuming $n = 3$, $K = 2.6 \times 10^{-5}$ M (if ACh carries 25% of the charge driven through the pipette), and $g_{max} = 18.3 \times 10^{-8} \Omega^{-1} \mu\text{m}^{-1}$. For comparison, the standard curves are shown for $n = 1, 2$ and 4 , respectively. In eight further experiments, K was almost constant (2.0×10^{-5} – 2.9×10^{-5} M), whereas g_{max} depended on the individual end-plate (6×10^{-8} – $20 \times 10^{-8} \Omega^{-1} \mu\text{m}^{-1}$), presumably as a result of variation in the density of junctional folds (0.5–3 per μm length)⁹.

The determination of n depends very critically on the assumption that the released ACh is proportional to charge passed through the pipette. But the value of n can also be obtained by a different experiment, independent of the pipette's properties. If a single pulse is released from the pipette, the measured time course of the conductance change can be compared with the calculated $g_{sum}(t)$, according to equation (3). This is done in Fig 2. The excellent coincidence of the theoretical curve ($n = 3$) with the measured response in both the declining and rising phase of end-plate current strongly supports the $n = 3$ conclusion of the dose-response curve. In addition, the successful description of the conductance time course justifies the earlier assumption of steady state between ACh concentration and receptor occupation in the experimental condition used. In other experiments the theoretical approximation was not as good as in the example illustrated, but in all cases the assumption of $n = 3$ clearly gave the best description.

Since the theoretical approximations of the experiments provided us with an average value of $g_{max} = 12 \times 10^{-6} \Omega^{-1} \mu m^{-1}$, we can calculate the number N of ionic channels per micrometre length of the terminal. Let γ be the conductance of a single channel, then $g_{max} = N\gamma$, since it is reasonable to assume that the closed time of the channel is short compared with the open time. The value of γ can be derived from measurements of ACh induced conductance fluctuations in the voltage clamped end-plate¹⁰⁻¹³. With $\gamma = 18.5 \times 10^{-12} \Omega^{-1}$ in the normal muscle fibres¹³ the density of ionic channels is, on the average, $6,500 \mu m^{-1}$. Since a terminal is about $2 \mu m$ wide and since receptors seem to be confined to the openings of the folds^{14, 15}, and possibly occur in patches^{9, 16-18} covering perhaps 50% of these regions, it is estimated that the 'active' surface is about $1 \mu m^2$ per micrometre length of terminal. Then the density of ionic channels is, on the average, $6,500 \mu m^{-2}$ at these regions of the postsynaptic membrane, a value quite close to observations of particle density in the respective patches obtained from freeze-etching studies^{9, 17}. Calculations of ACh-binding sites based on measurements by autoradiography^{14, 15, 19} are within this range.

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Turnover of junctional and extrajunctional acetylcholine receptors of the rat diaphragm

THE junctional acetylcholine receptors on the motor end-plate mediate the transmission of nerve impulses to skeletal muscle, and their number per endplate is normally kept constant at $1.4-4.7 \times 10^7$ for various vertebrate muscles¹⁻⁴. In some conditions, as in rats chronically treated with neostigmine^{5, 6} or in myasthenia gravis⁶, the number of acetylcholine receptors is markedly decreased, whereas, like denervation, insufficiency of neuromuscular transmission caused by various blocking agents, such as hemicholinium-3 and β bungarotoxin, tends to increase the number of receptors both at end-plate and non-end-plate zones (C. C. C., S. T. Chuang and M. C. H., unpublished). We, therefore, examined the turnover of acetylcholine receptors at end-plate and non-end-plate zones (extrajunctional) to shed more light on the pathophysiology of skeletal muscle.

The specific, and apparently irreversible, binding of α bungarotoxin isolated from the venom of *Bungarus multicinctus*⁷ made this study feasible. To bind selectively the receptor of the diaphragm without killing the animal, a

sublethal dose ($150 \mu g kg^{-1}$) of 3H -diacetyl α bungarotoxin⁸ dissolved in 1 ml normal saline was injected into the thoracic cavity of Long Evans rats (body weight about 220 g) and the animals held in upright position for several hours. Both hemidiaphragms were isolated between 4 h and 5 d after toxin-injection, washed for 3 h with Tyrode solution at $37^\circ C$ to remove the non-specific binding, cut parallel to the end-plate zone into three segments and the radioactivity in the central segment containing end-plates and the other two segments (non-end-plate) counted as previously described⁹. No difference was found in the binding of labelled toxin between both sides of hemidiaphragms. In the diaphragms isolated 4-9 h after injection of toxin, all the acetylcholine receptors existing on the end-plate were bound with the toxin, as further treatment of the muscle *in vitro* with $1 \mu g ml^{-1}$ of the labelled toxin for 2 h at $37^\circ C$, a condition known to saturate the receptors⁴, did not increase the binding of labelled toxin. As illustrated in Fig. 1, a sub-

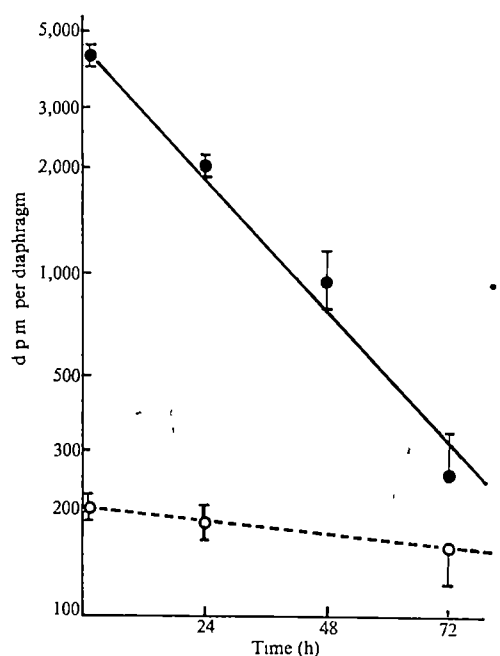


Fig. 1 The decline in 3H - α bungarotoxin bound to junctional and extrajunctional acetylcholine receptors. 3H - α bungarotoxin ($150 \mu g kg^{-1}$) was injected into the thoracic cavity of normal or denervated (9 d) rats. Radioactivity (d.p.m.) in the motor endplate zone of the diaphragm (junctional) is shown for the normal animal (O) and that in the whole muscle fibre (extrajunctional, counts on the end-plate subtracted) is shown for the denervated rats (●) at various times after injection of the toxin. The values at zero time were obtained by saturating the receptors *in vitro* with the labelled toxin. Mean \pm s.e. ($n = 6-9$).

stantial amount of labelled toxin remained bound to receptors 1-3 d after injection of toxin. It is obvious that the end-plate radioactivity declined exponentially at a very slow rate (half-time calculated as 7.5 d). The radioactivity in the serum, 9 h after injection of toxin, was very low and treatment of normal diaphragms with this serum did not show appreciable binding on the end-plate area. It is therefore unlikely that the slow decay of end-plate radioactivity was caused by continuous binding of circulating toxin with free acetylcholine receptors. The question arises whether the decline of radioactivity indeed resulted from turnover of the junctional receptors, from metabolic decomposition of the bound toxin, or from dissociation of the toxin from the receptor. Although the action of α bungarotoxin is considered practically irreversible, the rate of dissociation may be so slow that it has not been detected in previous studies.

Actinomycin D (0.5 mg kg^{-1}) was therefore administered simultaneously with the labelled toxin. This treatment depressed the generation of extrajunctional receptors in the rat diaphragm after denervation but not the binding of toxin to the existing receptors in the motor end-plate*. Interestingly, more radioactivity was found 5 days after toxin plus actinomycin D injection than in the untreated control (Table 1), indicating that the decline of radioactivity was retarded. Although this small inhibition by actinomycin D may not be sufficient to rule out the possibility of slow dissociation of toxin from the receptor, it does suggest that the decline in radioactivity may result partly from turnover of the junctional receptors. In other experiments, a motoneurone blocking agent, β bungarotoxin* ($50 \mu\text{g kg}^{-1}$), was injected together with the labelled α bungarotoxin or the phrenic nerve was cut immediately before administration of the labelled toxin. As shown in Table 1, the radioactivity remaining 5 days after labelling was less than in the control. Since both denervation^{1,4} and treatment with β bungarotoxin (CCC and MCH, unpublished) markedly increase the synthesis of new acetylcholine receptors, the more rapid decline of radioactivity in these conditions may be related to an increased turnover.

Table 1 Effects on the decline of ^3H - α bungarotoxin bound to junctional acetylcholine receptors

	Radioactivity (d.p.m. \pm s.e.)		
	Central segment (A)	Outer segment (B)	End-plate (A-B)
Control ($n = 7$)	98.5 \pm 8.7	32.8 \pm 4.0	64.9 \pm 8.0
Actinomycin D ($n = 4$)	102.9 \pm 7.7	1.9 \pm 1.8	100.0 \pm 7.9*
β bungarotoxin ($n = 5$)	61.4 \pm 10.4	28.8 \pm 7.1	33.1 \pm 4.7*
Denervation ($n = 4$)	91.4 \pm 2.9	51.8 \pm 4.9	40.2 \pm 4.4*

The labelled toxin was administered as described in Fig. 1 and the radioactivity remaining bound was measured 5 d after injection. Actinomycin D (0.5 mg kg^{-1}) or β bungarotoxin ($50 \mu\text{g kg}^{-1}$) was given together with the labelled toxin. For denervation, the left phrenic nerve in the neck was cut just before injection of toxin.

* $P < 0.05$ against control.

If one assumes that an acetylcholine receptor occupied by α bungarotoxin has the same half-life as an intact receptor, one may take the half-time of disappearance of labelled toxin from the end-plate segment as the turnover of the junctional receptors. The value of 7.5 d obtained in this experiment, however, could be an underestimate since the decline in radioactivity may result partly from dissociation of the toxin as well as from metabolism. The decrease in number of junctional receptors to about half within 7 d in rats chronically treated with neostigmine and the subsequent restoration to normal in 5 d after withdrawal of the anticholinesterase⁵, suggest that the turnover of receptors may be varied under altered neuromuscular transmission.

The turnover of extrajunctional receptors was studied by examining the receptors newly generated on the diaphragm muscle after sectioning of its phrenic nerve. After 9 d of denervation, when the receptor number almost reached maximum⁶, the rats were injected with the labelled toxin as described above for the normal animal. The toxin-binding sites of the denervated diaphragm at the time of injection was determined by treating diaphragms *in vitro* with $1 \mu\text{g ml}^{-1}$ of the labelled toxin for 2 h at 37°C . The radioactivity which remained bound to the whole denervated diaphragm 1–3 d after labelling is shown in Fig. 1. In contrast to the junctional receptors, the decline in radioactivity in the extrajunctional regions occurred at a much more rapid rate. The half-time was only 19 h. Berg and Hall¹⁰ reported a half-time of about 8 h in cultured diaphragms denervated for 4 days and the decline was inhibited by cycloheximide added to the culture medium. The shorter half-time in their experiment could result either from the

difference in turnover *in vivo* and *in vitro* or the different time of experiment after denervation.

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Effects of 'calcium ionophore' X537A on frog skeletal muscle

CALCIUM ions are critically involved in the cholinergic neuromuscular transmission process. Presynaptically, Ca^{2+} is required in transmitter release¹ while postsynaptically, it plays a role in acetylcholine-receptor kinetics². Furthermore, Ca^{2+} sets off a chain of physicochemical events which leads to muscle contraction³.

The carboxylic antibiotic X537A (ref. 4) has been applied to a number of Ca^{2+} -dependent biological systems (see ref. 6 for review). The important feature of such investigations is that X537A can complex divalent cations and thereby facilitate their diffusion through organic solvents⁵ and black lipid membranes⁶. For these reasons, X537A has been used to study the role of Ca^{2+} in neuromuscular systems. Levy *et al.*⁷ have shown that X537A, when applied to rat diaphragm, results in an increase in resting and twitch tensions, which they correlated with X537A-induced release of Ca^{2+} from the sarcoplasmic reticulum (SR). Kita and Van der Kloot⁸ have shown that X537A causes transient increases in the amplitudes of end-plate potentials and in the frequency of miniature end-plate potentials in frog skeletal muscle. These results were explained on the basis of an X537A-facilitated Ca^{2+} influx into the presynaptic nerve terminals.

As a part of our investigation of the role of Ca^{2+} in the permeability-controlling mechanism in the postjunctional membrane of frog skeletal muscle, we have made a preliminary study of the effects of X537A on resting potentials (RPs) and directly initiated action potentials (APs) in muscle fibres. Our results indicate that contrary to expectation the principal cation transported by X537A across muscle fibre membranes is Na^+ rather than Ca^{2+} .

Using established electrophysiological recording techniques, RPs and APs were obtained from frog (*Rana pipiens*) cutaneous pectoris muscles (Table 1). Experiments 1–4 show that exposure of muscles to X537A results in a 10 mV reduction in the RP. This is a steady-state condition which can be maintained for at least 2 h. Under these conditions the amplitudes of the APs are reduced by 25–30 mV, while generally their durations (as measured across the -20 mV line) are prolonged. The critical membrane potential, E_{crit} , is not significantly altered. Substitution of choline⁺ for Na^+ in the medium bathing the X537A-treated muscles results in an immediate membrane hyperpolarisation beyond control values. No APs could be generated by direct stimulation, which provides further evidence that the membrane is impermeable to choline⁺.

When, after exposure to X537A, choline⁺-substituted Ringer solution is in turn replaced with the normal Na^+ Ringer solution, the muscle fibres return to a depolarised state in which APs of reduced amplitudes can again be elicited. Figure 1 shows a typical set of recordings made from muscles bathed

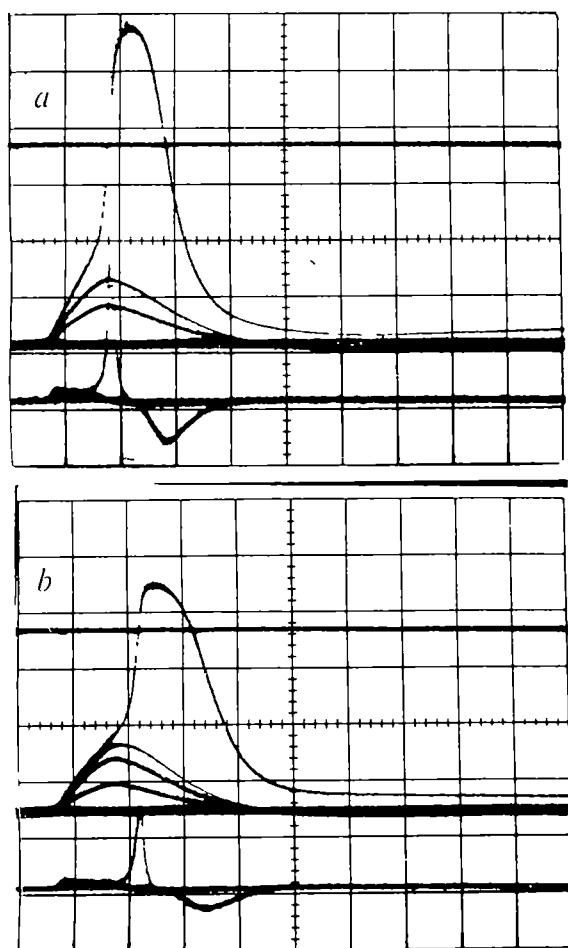


Fig. 1 Typical action potentials recorded from directly stimulated frog cutaneous pectoris muscle (stimulus duration = 1 ms). Lower traces show rate of change of membrane potential (dV/dt). Ordinate calibration, upper trace = 25 mV per division, lower trace = 250 mV per division. *a*, Control, muscle bathed in Ringer solution, first two stimuli were subliminal. *b*, Muscle bathed in Ringer solution, after 10 min exposure to $14 \mu\text{M}$ X537A, first three stimuli were subliminal.

in HEPES-Ringer solution before and after treatment with $14 \mu\text{M}$ X537A.

The choline⁺-substitution experiments provide strong evidence that an X537A-mediated Na^+ influx is responsible for the observed reduction in RP. An additional experiment was carried out to test the possibility that both Na^+ and Ca^{2+} influxes may be responsible for the depolarisation of the X537A-treated muscle fibres. In this experiment the bathing solution contained Ca^{2+} in increased concentration (composition: 108.8 mM Na^+ , 2.5 mM K^+ , 3.6 mM Ca^{2+} , 117.1 mM Cl^- , $3.0 \text{ mM HEPES buffer}$, $\text{pH} = 7.4$). Table 1 (experiment 5) shows that, with increased Ca^{2+} concentration the control values for E_{crit} became less negative. The higher Ca^{2+} concentration, however, did not significantly alter the previously observed effects of X537A on the RPs and APs. We concluded therefore that any increase in Ca^{2+} flux caused by X537A cannot be a significant factor in producing the observed membrane depolarisation.

The modest reduction in RP produced under our experimental conditions is not sufficient to account for the altered AP waveform which occurs in X537A-treated fibres. The changes in the AP may be explained by a specific interaction between X537A and the voltage-dependent Na^+ and K^+ channels in the muscle fibres. Tetrodotoxin (TTX) interacts with the voltage-dependent Na^+ channels, inhibiting the conductance change which underlies the generation of the muscle AP but does not alter the muscle RP (ref. 9). Therefore, we have carried out a TTX-X537A 'competition' experiment to determine, at least in resting fibres, whether or not X537A interacts with the voltage-dependent ionic channels.

Resting potentials and APs were obtained for a muscle bathed in the following sequence of solutions: (1) normal HEPES-Ringer solution (controls), (2) HEPES-Ringer solution plus $0.32 \mu\text{M}$ TTX, (3) $15 \mu\text{M}$ X537A in the $0.32 \mu\text{M}$ TTX-Ringer solution, applied for 10 min and then replaced with $0.32 \mu\text{M}$ TTX-Ringer solution. The RPs recorded for this sequence ($n = 10$ fibres for each step) were (1) $-93.2 \pm 0.9 \text{ mV}$ in control solution, (2) $-94.0 \pm 0.9 \text{ mV}$ in TTX-Ringer solution before X537A treatment, (3) $-79.4 \pm 1.3 \text{ mV}$ in TTX-Ringer solution after exposure to X537A. APs could not be initiated by direct stimulation in the presence of TTX before, during, or after exposure to X537A. By comparing the RPs obtained in these experiments with those given in

Table 1 Resting potentials and action potential characteristics for frog cutaneous pectoris muscles exposed to X537A

Exp †	n^*	RP (mV)†	E_{crit} (mV)‡	OS (mV)§	MRR (mV s ⁻¹)¶	MRF (mV s ⁻¹)	Duration (ms)**
Controls in HEPES-buffered (Na^+) Ringer							
1	6	-90.8 ± 0.8	-53.4 ± 0.9	51.3 ± 1.1	621.7 ± 11.4	180.8 ± 4.6	0.93 ± 0.02
2	8	-93.4 ± 0.9	-51.4 ± 0.6	47.3 ± 0.9	661.7 ± 6.5	140.0 ± 3.6	1.30 ± 0.02
3	10	-91.0 ± 1.0	-53.3 ± 0.6	52.5 ± 1.1	667.1 ± 18.2	185.0 ± 3.8	1.19 ± 0.02
4	9	-91.0 ± 0.6	-52.0 ± 0.6	50.8 ± 1.1	562.0 ± 3.7	163.0 ± 4.9	1.37 ± 0.00
5††	10	-90.4 ± 0.8	-45.2 ± 0.6	46.6 ± 0.9	603.3 ± 13.6	139.4 ± 2.7	1.20 ± 0.01
In HEPES-buffered (Na^+) Ringer, after 10 min in $14 \mu\text{M}$ X537A solution							
1	8	-80.5 ± 1.2	-52.8 ± 1.2	23.1 ± 1.4	348.8 ± 23.1	76.2 ± 11.5	1.23 ± 0.08
2	6	-82.3 ± 2.8	-49.6 ± 1.4	22.2 ± 0.6	417.5 ± 20.2	108.0 ± 5.8	1.26 ± 0.05
3	8	-81.4 ± 0.7	-53.3 ± 0.8	22.9 ± 1.0	395.7 ± 19.7	114.3 ± 5.7	1.27 ± 0.02
4	10	-77.9 ± 0.9	-48.6 ± 0.9	18.2 ± 0.9	286.2 ± 16.9	62.5 ± 1.9	1.78 ± 0.04
5***	16	-79.3 ± 0.8	-42.4 ± 0.5	22.0 ± 1.0	348.2 ± 10.0	64.0 ± 1.9	1.46 ± 0.02
In choline ⁺ -substituted Ringer							
1	7	-97.7 ± 1.1	—	—	—	—	—
2	10	-94.2 ± 0.9	—	—	—	—	—
3	14	-95.9 ± 1.0	—	—	—	—	—
4	10	-93.5 ± 1.1	—	—	—	—	—

*Number of records, †resting potential, ‡critical membrane potential, §overshoot of AP, ¶maximum rate of rise of AP, ||maximum rate of fall of AP, **duration of AP, measured at -20 mV line.

††Experiment 5 conducted in the presence of high (3.6 mM) extracellular Ca^{2+} throughout.

*** $15 \mu\text{M}$ X537A was used.

Muscles were bathed in HEPES-buffered Ringer solution (composition: 112.4 mM Na^+ , 2.5 mM K^+ , 1.8 mM Ca^{2+} , 117.1 mM Cl^- , $3.0 \text{ mM HEPES buffer}$, $\text{pH} = 7.4$). The muscles were then bathed for 10 min in HEPES-buffered Ringer solution containing $14 \mu\text{M}$ X537A (the X537A was first solubilised in ethanol). The X537A solution was then removed and the muscle washed several times with HEPES-Ringer solution to remove the ionophore from the bath. Thereafter, with the muscle bathed in HEPES-Ringer solution, RPs and APs were again determined. Finally, Na^+ -free choline⁺-Ringer solution (composition: $112.4 \text{ mM choline}^+$, 2.5 mM K^+ , 1.8 mM Ca^{2+} , 117.1 mM Cl^- , $3.0 \text{ mM HEPES buffer}$, $\text{pH} = 7.4$) was substituted as the bathing medium and RPs were determined.

Table 1, it can be seen that the effect of X537A on the RP is independent of the presence of TTX. If the RPs in fibres pretreated with TTX had remained unaffected by X537A, we could have concluded that X537A alone alters the RP by changing the resting behaviour of the voltage-dependent Na^+ channels. On the other hand, had we been able to generate APs in fibres treated first with TTX and then X537A, we could have concluded that X537A competes for the same active site which TTX inhibits. Neither of these postulated results was observed. It is therefore likely, although not certain, that X537A does not interact with the voltage-dependent Na^+ channels which TTX inhibits. Instead, X537A probably provides a separate, voltage-independent Na^+ conductance.

Our finding that X537A enhances resting Na^+ influx is supported by the work of Cornelius *et al.*¹⁰, who have published values for the binding constants of various cations to X537A in ethanol. The binding constants for Na^+ and Ca^{2+} are given as $1.5 \pm 0.7 \times 10^6$ and $2.7 \pm 1.5 \times 10^5$, respectively. Thus Na^+ is preferentially bound to X537A by roughly one order of magnitude. The ability of an ionophore to facilitate ionic diffusion generally follows its ability to form a complex with that ion.⁵

In view of our findings, it may be useful to re-examine the conclusions reached in earlier investigations using X537A. If this ionophore were depolarising the rat diaphragm muscles studied by Levy *et al.*⁷ to values below E_{crit} , the contractile apparatus could be activated and tension increased without any direct action of the X537A on the SR. Similarly, depolarisation of the presynaptic nerve terminals by means of an X537A-facilitated Na^+ influx could in part account for the rapid, transient release of acetylcholine observed by Kita and Van der Kloot.⁸

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The mechanism of calcium ionophore-induced secretion from the rat neurohypophysis

THE release of neurohypophyseal hormones, whether evoked by electrical stimulation or by a high potassium concentration, is associated with an uptake of calcium from the extracellular space.¹⁻⁴ It has therefore been proposed that calcium entry plays a key role in stimulus-secretion coupling in the neurohypophysis.⁵⁻⁷ Recently, calcium ionophores have been described which increase calcium flux across the cell membrane and induce secretion in a number of secretory systems.⁸⁻¹² In contrast, we report that although the calcium ionophore X-537A (Lasalocid, Hoffmann-LaRoche) promotes secretion from the rat neurohypophysis, there is no corresponding increase in calcium uptake; furthermore, ionophore-induced secretion

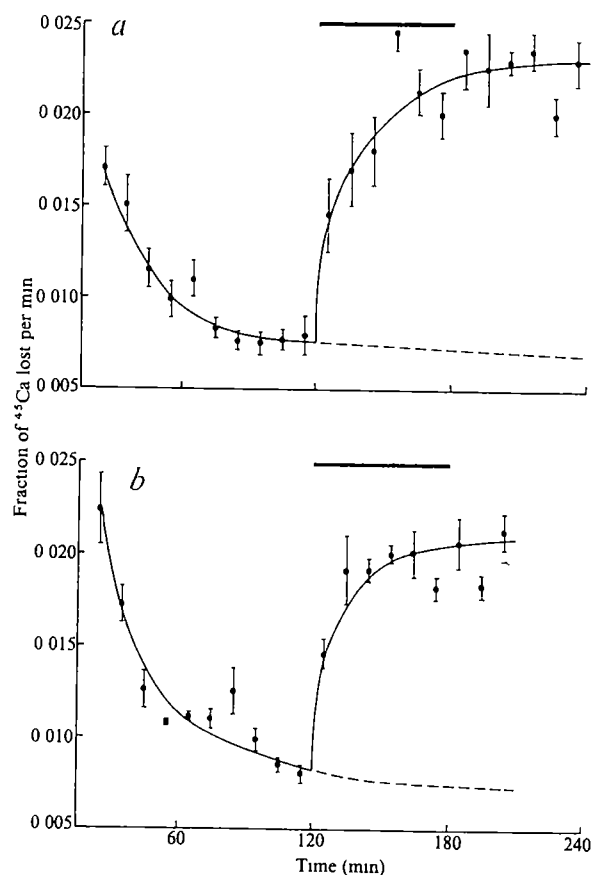


Fig. 1 Effects of X537A on the efflux of calcium from groups of four isolated neurohypophyses. The efflux was performed in a normal Locke solution (a) and in a calcium-free solution (b). X-537A ($20 \mu\text{g ml}^{-1}$) was added to the perfusate as indicated by the bars. Each point represents the mean rate constant ($\pm \text{s.e.m.}$, $n_a = 8$, $n_b = 4$) of calcium efflux observed during 240 min of washout (b, 210 min) starting at time zero. The dashed lines represent the control experiments. The bathing solution contained: NaCl , 150 mM; KCl , 5.6 mM; CaCl_2 , 2.2 mM; MgCl_2 , 1 mM; NaHCO_3 , 6 mM; glucose 10 mM. Dimethylsulphoxide (1%) was present throughout all efflux experiments. Neurohypophyses were isolated from albino rats (350-400 g body weight) and incubated at 37°C in 1 ml oxygenated Locke solution.¹ After preincubation for 20 min both control and test groups of glands were transferred for 10 min to a Locke solution containing dimethylsulphoxide (1%). The glands were then exposed to the same solution containing radioactive calcium ($10 \mu\text{Ci ml}^{-1}$) for 30 min. The ionophores, diluted in dimethylsulphoxide, were present at a concentration of $20 \mu\text{g ml}^{-1}$ during the last 25 min of exposure to the label. To remove the extracellular radioactive calcium both test and control groups of glands were washed for 60 min in a normal Locke solution, changed every 10 min. The glands were then blotted, weighed, digested overnight in solene and the ^{45}Ca content counted. The oxytocin released into the incubation medium was determined by bioassay¹³ confirming the results already obtained.¹² To study the efflux of ^{45}Ca from the neurohypophysis, groups of four glands were loaded for 60 min in a solution containing radioactive calcium ($33 \mu\text{Ci ml}^{-1}$) and then transferred to a solution containing non-radioactive calcium. After 10 min the solution was removed, replaced by a fresh solution and counted. This sequence was repeated for up to 240 min. Dimethylsulphoxide (1%) was present throughout all the efflux studies. At the end of the experiment the glands were digested with solene and the ^{45}Ca counted. When sodium chloride was removed from the solution, choline chloride was added to maintain isotonicity.

persists in the absence of external calcium ions. The most logical explanation for these results is that, rather than increasing the influx of extra-cellular calcium, the ionophore actually releases calcium from intracellular binding sites, probably mitochondria, and that it is the mobilisation of this internal calcium which, *inter alia*, promotes secretion.

Of the two ionophores tested, A23187 (Lilly) had no effect

Table 1 Effects of calcium ionophores in the neurohypophysis on calcium uptake

Experiment	^{45}Ca uptake (c.p.m. mg^{-1})
Control	443 ± 48 (8)
X537A	197 ± 21 (8)
Control	442 ± 54 (4)
A23187	410 ± 40 (4)

on either secretion¹² or calcium uptake, whereas X537A increased secretion¹² but reduced calcium uptake (Table 1). One possible explanation for this rather surprising observation is that X537A enters the cells and releases calcium from intracellular binding sites. Evidence favouring this interpretation is shown in Fig 1. Application of X537A increases markedly the efflux of ^{45}Ca from the neurohypophysis. This may arise because X537A increases some form of internal calcium exchange either for external calcium or for external sodium ions, or because X537A raises the intracellular level of ionised calcium and this in turn is reflected in a higher rate of calcium loss from the cell. This latter interpretation seems most likely because the ionophore-induced calcium efflux persists in the absence of external calcium and sodium ions and in four experiments was still observed when ionophore was added in a solution lacking sodium, calcium and magnesium ions. Although part of the decrease in ^{45}Ca influx could be explained by a persistent increase in Ca efflux after removal of ionophore, it is possible to calculate from the efflux rate constants, that at least 37% of the observed diminution in ^{45}Ca influx must be attributed directly to ionophore action.

These data are fully consistent with the idea that the important step in initiating secretion is not simply an increased rate of calcium entry but an increase in internal ionised

calcium concentration, and that X537A promotes just such a rise by releasing calcium from some internal binding site. As much of the intracellular calcium in nerve cells is membrane-bound within mitochondria, these organelles are an obvious candidate as the site of action for X537A. Figure 2 shows experiments designed to test whether calcium ionophores have any effect on calcium binding by mitochondria isolated from brain. In the presence of X537A and A23187 calcium uptake is reduced and calcium efflux is increased. If these effects also occur in intact cells, they would clearly lead to an increase in ionised calcium at the expense of calcium sequestered in the mitochondria.

These results, therefore, confirm the idea that an essential step in the stimulation-secretion coupling mechanism is an increase in the internal ionised calcium concentration. Hence the effect of X537A on neurohypophyseal secretion seems analogous to that in which intracellular microinjection of calcium into nerve terminals induces an increase in the quantal release of neurotransmitters¹⁴. Finally, our results highlight the need for care in interpreting changes produced by calcium ionophores as resulting solely from a modification of the permeability of the surface membrane to calcium.

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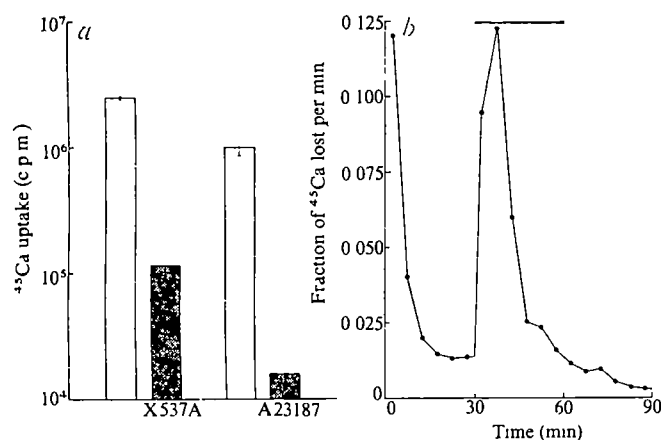


Fig 2 Effects of calcium ionophores on calcium fluxes from isolated brain mitochondria. *a*, Uptake of ^{45}Ca into mitochondria isolated from guinea-pig brain cortex¹⁵. The preparation was kept in a solution containing a calcium ionophore for 3 min. ^{45}Ca was present during the last minute of exposure to the ionophore. Mitochondria were then washed on a Millipore filter and counted. White columns, control uptake (X537A, control, $n = 4$, A23187, control, $n = 4$). Shaded columns, calcium uptake in the presence of X537A ($20 \mu\text{g ml}^{-1}$, $n = 4$) and A23187 ($20 \mu\text{g ml}^{-1}$, $n = 4$). Each column represents the mean \pm s.e.m. *b*, Efflux of ^{45}Ca from brain mitochondria. The preparation was exposed to the label for 30 min and subsequently placed on a Millipore filter. The efflux of ^{45}Ca was determined by exposing the mitochondria to a buffer solution for 5 min followed by collecting the solution under negative pressure. This sequence was repeated at intervals of 5 min for 90 min. The amount of ^{45}Ca was measured in each sample. In addition the mitochondrial ^{45}Ca content was determined at the end of each experiment. Heavy bar, time of exposure to X537A ($20 \mu\text{g ml}^{-1}$). Buffer solution contained KCl, 100 mM, MgCl_2 , 10 mM, Tris maleate, 20 mM, succinate, 5 mM, KH_2PO_4 , 4 mM, ATP, 2 mM, CaCl_2 , 100 μM . It was equilibrated with 5% CO_2 in O_2 .

Functional inactivation of bacteriophage λ morphogenetic gene mRNA

THE synthesis of the morphogenetic proteins of bacteriophage λ seems to be regulated at the post-transcriptional level. This conclusion is based on the observation that the ratio of protein to DNA along the left arm of the λ genome varies from gene to gene by as much as 870-fold (Fig 1), while the ratio of mRNA to DNA in this region varies less than twofold¹, reflecting its transcription from a single promoter as a polycistronic mRNA^{2–5}. These large variations in the molar ratios of the morphogenetic proteins could be explained by three different control mechanisms: (1) The initiation of protein synthesis could be controlled at the level of ribosome binding either by initiation factor complexes or by mRNA secondary structure^{6,7}. (2) Some morphogenetic proteins could act to regulate their own translation or that of neighbouring genes (N. Sternberg, personal communication). (3) The differential translation of the late gene transcripts could be achieved by selectively inactivating some transcripts but not others. Our previous experiments using RNA-DNA hybridisation ruled out differential chemical decay of the mRNA derived from the late region of the genome, but did not exclude the possibility that differential functional inactivation of morphogenetic gene transcripts could account for

the late protein to DNA variation¹. Recent experiments indicate that functional and chemical decay are different processes which can vary widely in rate⁸ and temperature dependence⁹. Functional decay in a polycistronic message generally seems to involve an endonucleolytic attack near the 5'-end of each gene transcript^{8,10} at a specific target either in or near the ribosome-binding site¹¹. Thus mRNA inactivation is not primarily a function of the length of a transcript and can vary from transcript to transcript within the same cell¹¹. Because of this variation, differential functional decay has been invoked as a possibly significant mechanism of post-transcriptional control^{11,12}.

In this report we examine the possibility that differential functional mRNA inactivation accounts for the post-transcriptional regulation of λ morphogenetic protein synthesis. To do this we have measured the functional half lives of individual late gene transcripts. We conclude that differential functional mRNA decay is not a significant factor controlling the translation of the morphogenetic genes of λ .

The rate of chemical decay of late λ mRNA was determined in a pulse-chase experiment by measuring the loss of labelled morphogenetic gene transcripts which hybridised to λ h⁺i⁸⁰ DNA as described in the legend to Fig 2a. The results shown in Fig 2a indicate that λ morphogenetic gene transcripts decay exponentially with an apparent half life of 2.5 min, after a 4-min lag.

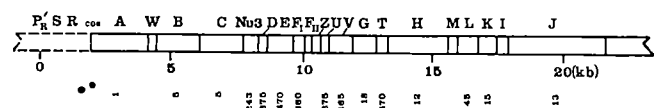


Fig 1 Physical and genetic map of the morphogenetic region of λ showing the relative number of copies of the different late proteins. The length of each gene and its position on the map is taken from Murialdo and Siminovitch²³ and Hendrix and Casjens²⁴. The morphogenetic genes are transcribed from left to right from the late promoter P_R⁷³. Map distances measured from the late promoter are given in kilobase pairs (kb). The numbers below each gene represent the relative number of copies of protein synthesised per cell, normalised to a value of 1 for the A protein. The absolute value for the A protein is estimated to be 320 molecules per cell²³.

This measured half life of 2.5 min is only slightly longer than the chemical half life for bulk *E. coli* mRNA^{12,16}, indicating that late λ mRNA is not unusually stable, as is the case of several other phage mRNAs including T7¹⁷, T4¹⁸, S13¹², M13¹⁹ and Φ X174²⁰. The reason for the 4-min delay in the onset of degradation is unclear. Because of the presence of an excess of unlabelled uridine and rifampicin during the chase, it seems unlikely that the lag represents a balance between the decay of pre-existing labelled RNA and the continued synthesis of newly labelled RNA. If, however, mRNA degradation involves primary endonucleolytic attack followed by secondary exonucleolytic hydrolysis, then one would expect a lag between the onset of degradation and the time at which the mRNA fragments become too small to form stable hybrids with λ h⁺i⁸⁰ DNA (ref 21).

This latter interpretation is consistent with existing evidence that late λ mRNA undergoes extensive endonucleolytic cleavage (D. Schlessinger, personal communication)^{2,4,5}.

The functional half life of late λ mRNA *in vivo* was measured by determining its ability to direct the synthesis of morphogenetic proteins after inhibiting transcription with rifampicin. The decrease in the rate of incorporation of ³⁵S-methionine at various times after addition of rifampicin 30 min after infection is shown in Fig 2b. In a control experiment in which rifampicin was omitted, the amount of ³⁵S-methionine incorporated in a 1-min pulse was relatively constant for at least 15 min, indicating that continued transcription was balanced by inactivation of mRNA. The addition of rifampicin perturbed this steady state,

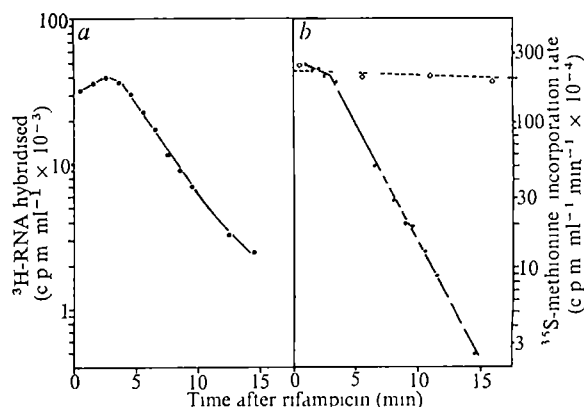
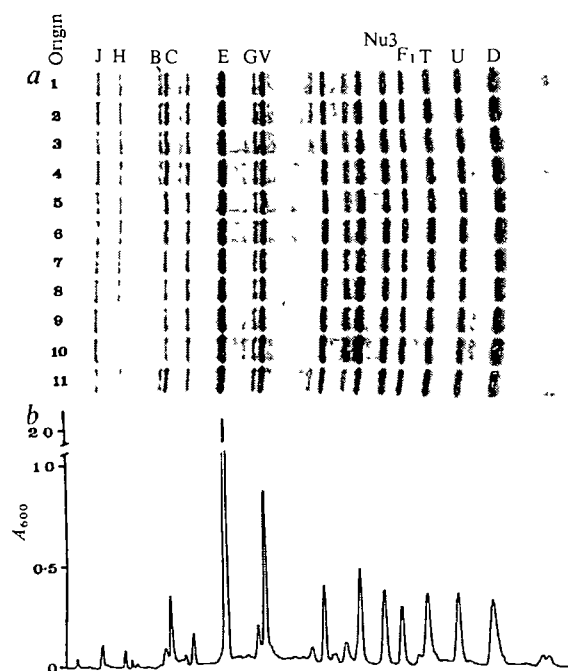


Fig 2 Chemical and functional decay of late λ mRNA. *a*, Chemical decay. A culture of *E. coli* K12 strain 159 hci⁻thy⁻sup⁻gal⁻ was grown to 5×10^8 cells ml⁻¹ in RM medium¹³. The cells were sedimented and resuspended in fresh RM medium at 2×10^8 cells ml⁻¹, ultraviolet-irradiated with 4,000 erg mm⁻² (254 nm), and infected with λ ct_{ts857}S_{am7} (multiplicity of infection of 5) at 0°C for 30 min¹³. The culture was diluted fivefold into warm (37°C) RM medium and incubated with aeration at 37°C. After 28 min nalidixic acid (30 μ g ml⁻¹) was added to depress DNA synthesis¹⁴. 5-³H-uridine (New England Nuclear Corp., 30 Ci mmol⁻¹) was added to a final concentration of 50 μ Ci ml⁻¹ at 30 min, and at 30.5 min labelling was stopped by the addition of rifampicin¹⁵ (Calbiochem) and unlabelled uridine to 500 μ g ml⁻¹ each. Samples (1 ml) of the culture were removed at 1-min intervals after addition of rifampicin and uridine, and the cells were lysed by adding sodium dodecylsulphate to 1% w/v and heating to 100°C for 2 min²⁵. The lysates were cooled to room temperature and deproteinised by two successive phenol extractions. The RNA was precipitated with cold ethanol, dissolved in 2 \times SSC and hybridised to an excess of λ h⁺i⁸⁰ DNA bound to nitrocellulose filters²⁶. Only RNA from the morphogenetic region of λ hybridises to this phage DNA²⁷. Approximately 90% of the c.p.m. incorporated was specific to the late region of λ , $t_{1/2} = 2.5$ min. *b*, Functional decay. A culture of strain 159 was ultraviolet-irradiated, infected, grown at 37°C as above and split into two portions. One portion was treated with rifampicin (500 μ g ml⁻¹) 30 min after infection and the other was not. The functional activity of the mRNA was determined by measuring the ability of the cells to synthesise proteins at various times after addition of rifampicin. Samples (1 ml) were withdrawn at various times and pulse-labelled for 1 min with ³⁵S-methionine (35 μ Ci ml⁻¹, New England Nuclear Corp., 200 Ci mmol⁻¹). Labelling was stopped by addition of unlabelled methionine (1 mg ml⁻¹) and sodium azide (50 mM). The cells were centrifuged and lysed by resuspending in electrophoresis sample buffer containing sodium dodecylsulphate and 2-mercaptoethanol and boiling for 1 min¹³. A control experiment (data not shown) indicated that 75% of the methionine incorporated was caused by λ infection. Diamonds, trichloroacetic acid (TCA) precipitable ³⁵S-methionine incorporated by the control culture in the absence of rifampicin. ●, TCA-precipitable ³⁵S-methionine incorporated by the culture after the addition of rifampicin. $t_{1/2} = 1.7$ min.

however, resulting in a biphasic decline in the rate of ³⁵S-methionine incorporation. For the first 4 min after rifampicin addition, the rate of methionine incorporation declined slowly, thereafter the rate declined rapidly with a half life of 1.7 min. The initial slow phase of decay was presumably because of a balance between the rate of mRNA inactivation and the rate of mRNA chain elongation by RNA polymerase molecules which had initiated transcription before the addition of rifampicin. These transcripts must have been completed within 4 min after the addition of rifampicin, when the decay became exponential.

To determine the functional half lives of the individual gene transcripts, the labelled samples from the above experiment were analysed by electrophoresis on SDS-polyacrylamide slab gels (Fig 3). The relative amounts of each of several morphogenetic proteins synthesised during the 1-min pulses were measured and plotted as a function of time after the addition of rifampicin (Fig 4a). All the curves had the same shape with a lag period of varying lengths followed by exponential decay.



The duration of the lag should reflect the time it takes an RNA polymerase molecule to transcribe the genes lying between the late promoter and the gene in question. If the rate of transcription is constant along the genome, then this lag is also a measure of the physical distance between the gene and the late promoter. When the lag time is plotted as a function of the distance from P_R' , we find that transcription ceases sooner than expected, especially for the distal genes *V* through *J* (Fig. 4b). The reason for the premature shut-off of transcription is not known. One possibility is that the rate of mRNA elongation is not uniform over this region of the genome. Another is that rifampicin might cause premature termination of transcription, thereby reducing the apparent delay of degradation for genes in this operon. As this effect is most obvious in the distal genes it may not have been apparent in previous studies with rifampicin which dealt with much shorter operons^{15,22}.

The functional half lives of the different morphogenetic transcripts determined from the exponential portion of the

Fig 3 Polyacrylamide gel electrophoretic pattern of λ morphogenetic proteins synthesised at different times after addition of rifampicin. *a*, Autoradiogram of a polyacrylamide gel ³⁵S-methionine-labelled samples from Fig 2 were analysed by SDS-polyacrylamide gel electrophoresis in slab gels containing 15% acrylamide in the separating gel²⁸. The TCA-precipitable counts loaded into each sample well were the same, the amount of material loaded increased with increasing time after addition of rifampicin as incorporation declined (compare Fig 2). The genes coding for the various morphogenetic proteins indicated by the letters have been identified previously^{21,24}. Sample wells 1 and 11 contained extracts of labelled cells from the control cultures not containing rifampicin labelled 30 min post-infection. The other wells contained extracts of cells labelled at the various times indicated after the addition of rifampicin, 2, 0.5–1.5 min, 3, 1–2 min, 4, 1.5–2.5 min, 5, 2–3 min, 6, 2.5–3.5 min, 7, 3–4 min, 8, 3.5–4.5 min, 9, 4–5 min, 10, 4.5–5.5 min. After electrophoresis the gels were dried and autoradiographed on Kodak SB-54 X-ray film. The gels on which the other samples of Fig 2 were analysed are not shown here. *b*, Densitometric scan of sample well 1 in the autoradiogram above. The autoradiograms from Fig 3a were scanned on a Gilford 2400 spectrophotometer equipped with a 20-cm linear transport. Care was taken to ensure that the optical density of the band was within the linear response of the film. The areas under individual peaks were measured by weighing to determine the relative amount of labelled protein in a particular band synthesised at different times after addition of rifampicin. Previous experiments have shown that approximately 95% of the radioactive protein resolved on the gel is phage-specific²⁸.

decay curves range from 1.1 min for *C* and *J* mRNAs to 1.9 min for *D* mRNA. Since functional inactivation of each transcript occurs at an exponential rate for at least 5–6 min, it seems most likely that this inactivation is the result of random endonucleolytic attack²². Because this decay shows no apparent correlation between mRNA half life and mRNA size, the decay process probably occurs at specific sites in the transcript, even though it is random in time. For example, genes *D* and *J* differ in size by a factor of 10 while their mRNA half lives vary by a factor of 2. Although it is possible that a single inactivation event at the 5'-end of the mRNA molecule could affect all genes simultaneously, the variation in the lag before exponential decay, which varies from gene to gene, suggests that this explanation is incorrect. We prefer a model in which individual transcripts are inactivated by independent events, such as endonucleolytic cleavages at the ribosome-loading sites.

The results presented here taken together with our previous results¹ indicate that there is no preferential chemical or func-

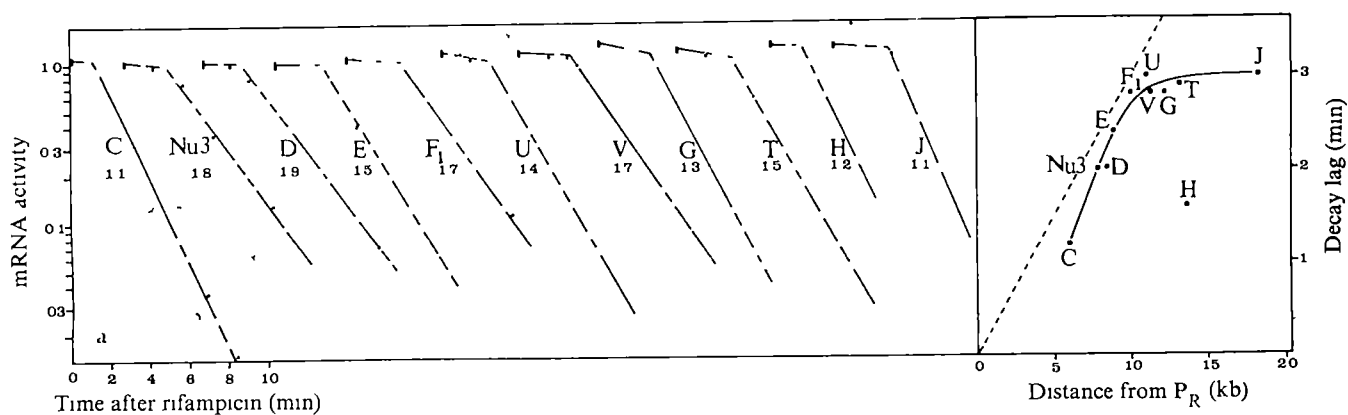


Fig 4 Functional instability of the individual morphogenetic gene transcripts. *a*, Kinetics of inactivation after addition of rifampicin. Results are expressed as the fraction of protein synthesised in a 1-min pulse by 1 ml of the rifampicin-treated culture relative to the untreated culture. The autoradiograms in Fig 3 were scanned and the relative amount of labelled protein in each band was measured at different times after rifampicin addition. The values obtained were corrected for the amount of sample loaded in each well of the gel. The curves for individual gene transcripts are presented in the same order as the map order and are displaced to the right to avoid confusion. The gene coding for each protein is indicated to the left of each curve together with the functional half life of the transcript in minutes. The half life was determined from the slope of the exponential portion of the curve. *b*, Lag time before exponential decay begins for each morphogenetic gene transcript. The duration of the lag phase of each curve in Fig 4a was plotted as a function of the distance in kilobase pairs of the left end of the gene from the late promoter P_R' (see Fig 1). The broken line represents the theoretical curve expected if the rate of RNA polymerase movement in the morphogenetic region of the genome is constant at 55 nucleotides per second²⁹.

tional decay of late λ mRNAs that could explain the large variation in the protein to DNA ratio seen in this region of the λ genome. Thus we believe that the synthesis of morphogenetic proteins is probably regulated at the level of ribosome loading mediated either by the mRNA primary or secondary structure, or by soluble factors which may be phage or host-coded.

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Control of gene expression in blue-green algae

THE blue-green algae are a major group of prokaryotes in many ways resembling, and perhaps phylogenetically related to, the chloroplasts of photosynthetic eukaryotes. Metabolic control mechanisms in this group are of interest because of its unique evolutionary position and because many of its members are obligate photoautotrophs and as such may show patterns of control different from those known in heterotrophic bacteria. Indeed, Carr and his collaborators¹⁻³ have proposed that blue-greens do not in general regulate metabolism at the level of gene expression, as heterotrophs commonly do. An exogenous metabolite may be assimilated, but its presence in the cell does not induce (or repress) the synthesis of enzymes responsible for its own catabolism (or biosynthesis). Thus, these workers argue, exogenous substrates do not usually support significant dark growth, or even stimulate growth in the light, because blue-greens simply cannot adjust the levels of the enzymes of intermediary metabolism to accommodate any source of carbon other than CO₂ or any source of energy other than light¹.

To establish whether any controls at the level of gene expression have evolved in blue-green algae, it seems pertinent to look for responses to environmental stimuli which do affect the growth of these organisms. Such stimuli include CO₂, sources of phosphorus and nitrogen, and, of course, light. Apparent induction or repression of enzyme systems responsible for assimilation of phosphate⁴, nitrate⁵ and

molecular nitrogen⁶ has been described. Here we show that removal of light provokes both quantitative and qualitative changes in the protein complement produced by the unicellular, obligately photoautotrophic blue-green alga *Anacystis nidulans*, and briefly discuss evidence for the 'dark induction' of two enzymes specifically required for dark endogenous energy metabolism.

Figure 1 shows the effect of removal of light on the rate

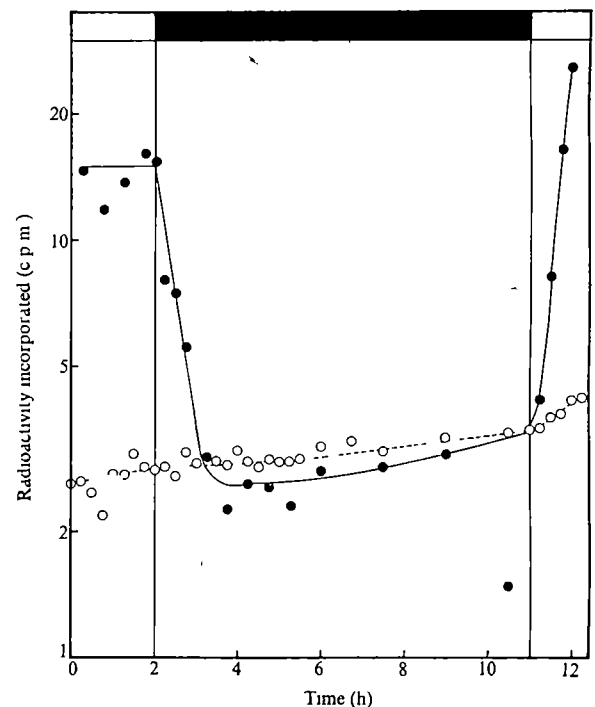


Fig. 1 Relative rates of protein synthesis in light and dark. A culture of our α -isopropylmalate synthetase deficient auxotroph, *leu201* (to be described elsewhere) was grown⁷ for 48 h (4-6 generations) in medium containing L-leucine at 10 $\mu\text{g ml}^{-1}$ and ¹⁴C-DL-leucine (New England Nuclear) at 0.01 $\mu\text{Ci ml}^{-1}$. Illumination was terminated at 2 h and resumed at 11 h. Samples of 1 ml were withdrawn at intervals to tubes containing 100 μCi ³H-L-leucine (New England Nuclear) and incubated for 15 min under the same illumination conditions as the culture. Labelling was terminated by addition of trichloroacetic acid to 5%, and radioactivity incorporated into protein determined as c.p.m. remaining acid precipitable after 20 min at 90°C. ●, ³H-leucine incorporated during 15 min pulses; ○, ¹⁴C-leucine incorporated during continuous labelling from -48 h.

of incorporation of radioactive leucine into protein in a leucine-requiring mutant of *A. nidulans*. As there is no endogenous leucine synthesis in this strain (unpublished) and little protein turnover (unpublished), label incorporation is a direct measure of protein synthesis. Rates fell (Fig. 1) during the first hour of darkness and remained low until the culture was reilluminated. At least a dozen proteins were synthesised, however, in the dark which in the light were either not made, or made in lower relative amounts. This is apparent from Fig. 2, which presents SDS-polyacrylamide gel profiles of soluble proteins from cultures labelled for two generations in the light with ¹⁴C-DL-leucine and then (after removal of ¹⁴C) with ³H-L-leucine either for 1 h in the light (a), during the first hour after darkening (b), between the fourth and tenth hour after darkening (c), or during the first hour after reillumination (d). Several polypeptides were prominent in dark-³H-labelled material (b and c) which were not prominent in either ¹⁴C-'prelabelled' or ³H-labelled material produced in the light (a and d). Most obvious were species of apparent molecular weights 90,000,

60,000, 48,000 and 41,000 (all values $\pm 10\%$) Eight additional preferentially synthesised polypeptide chains could be identified when the ratios of ^3H to ^{14}C in each gel slice were plotted, as in Fig 3 Two of these (130,000 and 29,000) were apparent only in material labelled during the first hour of darkness Ratios of ^3H to ^{14}C were relatively constant when ^3H -leucine incorporation occurred in the light (a and d), although there was some evidence for preferential synthesis of material of very high molecular weight immediately after reillumination of darkened cells (d) All these patterns are reproducible, with some variation in peak height from experiment to experiment Addition of the photosystem II inhibitor 3-(p-chlorophenyl)-1,1-dimethylurea (CMU) produced an alteration in the pattern of protein synthesis grossly similar to that provoked by darkness

These data do not allow us to assign functions to any of the polypeptide chains produced preferentially in the dark It is reasonable to assume that at least some of these are enzymes (or subunits of enzymes) specifically required in the dark It is known that certain enzymes of the oxidative pentose phosphate pathway are essential only for dark (endogenous) metabolism^{8,11} We therefore measured specific activities of three of these (glycogen phosphorylase, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase) in cultures before and after removal of light

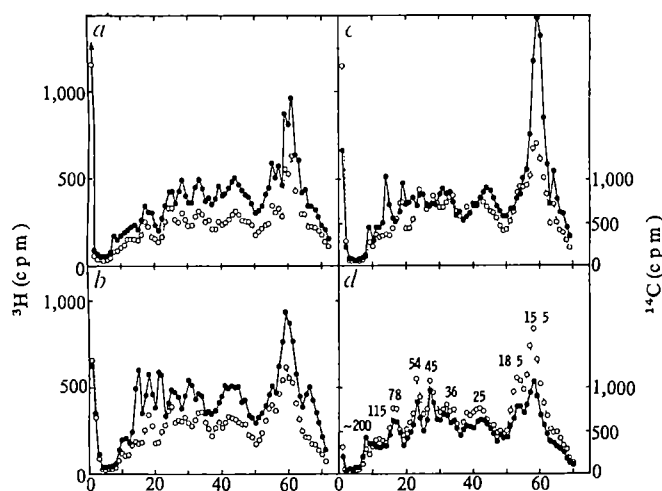


Fig 2 Sodium dodecylsulphate (SDS)-polyacrylamide gel profiles of soluble proteins from cells labelled in light and dark A culture of *leu201* was grown for 15 h in medium containing $10 \mu\text{g L-leucine ml}^{-1}$ and $^{14}\text{C-DL-leucine}$ at $0.1 \mu\text{Ci ml}^{-1}$ The culture was collected, washed, and resuspended in non-radioactive, leucine-containing medium After 90 min growth to allow recovery, the culture was divided into four subcultures Subculture a was exposed to $^3\text{H-L-leucine}$ at $0.8 \mu\text{Ci ml}^{-1}$ for 1 h in the light, while subcultures b, c and d were darkened b Was labelled ($3.8 \mu\text{Ci } ^3\text{H-L-leucine ml}^{-1}$) during the first hour after darkening, c was labelled ($3.8 \mu\text{Ci } ^3\text{H-L-leucine ml}^{-1}$) between the fourth and tenth hour after darkening, d was reilluminated after 9 h and labelled ($0.8 \mu\text{Ci } ^3\text{H-L-leucine ml}^{-1}$) during the first hour after reillumination Each subculture was collected by centrifugation immediately after labelling, resuspended in lysis buffer^{8,11} and passed through an Aminco French pressure cell at 16,000 pounds inch^{-2} Lysates were centrifuged at $17,000g$ for 30 min and protein was precipitated from the supernatant fluids with trichloroacetic acid at 5% Precipitates were collected by centrifugation, washed twice with ethanol, dried and solubilised in sample buffer⁹ by heating at 100°C for 10 min Samples were loaded on and electrophoresed through 7.5% polyacrylamide gels containing SDS⁹ Gels were sliced, solubilised in Protosol, and counted in toluene-Omnifluor (New England Nuclear) for determination of ^{14}C and ^3H radioactivity Numbers in d indicate molecular weights (in thousands) determined with reference to a set of standard marker proteins run on parallel gels¹⁰ Qualitatively similar profiles were obtained when total lysate proteins, rather than $17,000g$ supernatant proteins, were treated in this way ●, ^3H -radioactivity, ○, ^{14}C -radioactivity

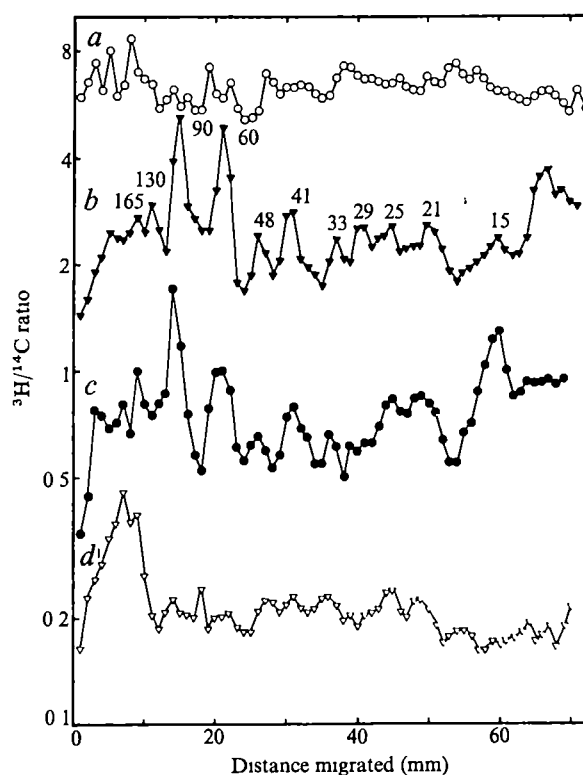


Fig 3 $^3\text{H}/^{14}\text{C}$ ratios The ratio of ^3H to ^{14}C radioactivity was computed for each slice of the gels represented in Fig 2 Numbers above curve b indicate molecular weights in thousands, as described for Fig 2 Ratios are plotted on a logarithmic scale to allow direct comparison of relative peak heights between gels

Although levels of the third enzyme remained constant, the first two showed reproducible, chloramphenicol-inhibitable, 1.5 and 2.0-fold increases (respectively) in specific activity These increases occurred over about one-third of a generation and at a period when overall rates of protein synthesis were rapidly falling (Fig 1) We calculate that they correspond to increases in the differential rates of synthesis of these two proteins of at least 20-fold (unpublished)

Although blue-green algae may indeed not respond to most exogenous substrates by altering levels of enzymes responsible for their utilisation or formation, we do not think it safe to maintain, as Carr¹ has done, that blue-greens are largely incapable of exerting control at the level of gene expression To demonstrate such control, however, it is necessary to look at environmental factors other than exogenous carbon compounds, and in particular at factors on which the growth of these organisms is known to depend

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Location of non-DNA components of closed circular colicin E1 plasmid DNA

COLICIN E1 plasmid (Col E1) DNA usually exists as closed-circular duplex molecules of molecular weight 4.2×10^6 (ref. 1). In addition to the form that is assumed to contain only deoxyribonucleotides linked by phosphodiester bonds, two other types of supercoiled closed-circular molecules have been described. One is a DNA-protein complex, called a relaxation complex². When the protein is denatured or destroyed, the complex is converted to an open-circular structure that has a break (a nick or a gap) in the heavy (H) strand (as defined by CsCl-poly(U,G) density gradient centrifugation)³. The other type is the closed-circular molecule that accumulates in bacteria when protein synthesis is inhibited and contains a sequence of ribonucleotides (RNA) generally in either the light or heavy strand^{4,5}. The functions of these non-DNA components in replication of the plasmid have been the subject of speculation^{2,5}. Recently, it was shown that Col E1 DNA replication initiates from a fixed region which is located approximately 20% of the molecular length from the single site of cleavage of endonuclease *Eco*R1⁶⁻⁸. Replication proceeds unidirectionally⁶⁻⁸ and terminates at or near the origin of replication⁹. It was thus interesting to locate the interruption in a DNA strand resulting from either the removal of the relaxation protein or the hydrolysis of the RNA with respect to the origin/terminus region. By measuring the size by gel electrophoresis of DNA fragments formed by denaturation of linear molecules produced by the treatment of open-circular molecules containing a relaxation break with endonuclease *Eco*R1, the break was located at approximately 20% of the length from the end of the linear molecule¹⁰. We developed an electron microscopic method to determine the position of a single-stranded break and found that the relaxation break is located approximately 20% of the length from the end which has the



Fig 1 Electron micrographs of relaxation complexes that had been treated successively with Pronase, exonuclease III and endonuclease *Eco*R1. Purified relaxation complexes (34S) were prepared² from A745 (Col E1) *thy* (ref. 9) grown in a medium containing ³H-thymidine (20 mCi mmol⁻¹). After treatment with Pronase (Sigma, 200 µg ml⁻¹) for 30 min at 37°C, the DNA (10 µg in 2 ml) was layered on 36 ml of a 15 to 50% linear sucrose gradient and centrifuged in a Beckman SW 27 rotor at 27,000 r.p.m. for 24 h at 20°C. The fractions containing open-circular molecules were pooled and centrifuged in a Beckman Type 40 rotor at 36,000 r.p.m. for 20 h at 15°C. The pelleted DNA was suspended in 0.1 M Tris, pH 8.0. After addition of MgCl₂ to 2 mM the DNA was treated with exonuclease III (provided by Dr M. Fuks) to render about 10% (a) or 25% (b) of the total amount acid-soluble. The reaction was terminated by the addition of EDTA to 4 mM. After inactivation of the enzyme by heating at 80°C for 10 min and addition of 10 mM of MgCl₂, the DNA was treated with *Eco*R1 (provided by Dr D. Nathans)⁶. Samples were prepared for electron microscopy⁶. The scale represents 1.0 µm.

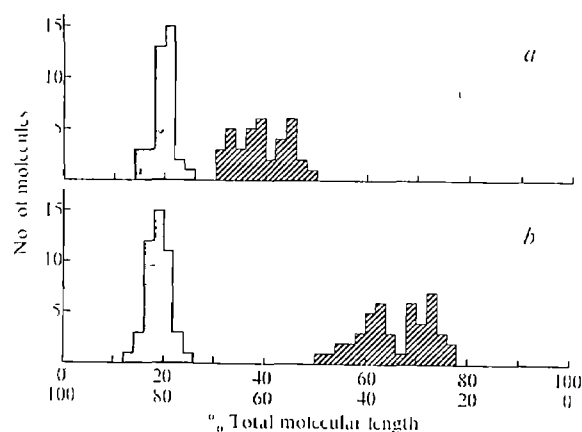


Fig 2 Histograms showing the distribution of the fractional lengths of two double-stranded regions of molecules in the samples described in the legend to Fig. 1. The result obtained with a, sample a, b, sample b. The length of the shorter double-stranded region (filled column) is shown by the fractional length from the left in the abscissa and that of the longer double-stranded region (hatched column) from the right. The average lengths of the shorter double-stranded regions of 37 molecules in sample a and 46 molecules in sample b are 0.45 ± 0.05 µm ($20.2 \pm 2.3\%$) and 0.43 ± 0.08 µm ($19.4 \pm 3.6\%$), respectively. The average length of the longer double-stranded regions of molecules in sample a is 1.37 ± 0.13 µm ($62.0 \pm 5.9\%$) and that in sample b is 0.77 ± 0.17 µm ($34.7 \pm 7.7\%$). The total length of untreated molecules is 2.22 ± 0.09 µm (100%).

3' end of the H strand of the linear molecule created by the treatment with endonuclease *Eco*R1. On the other hand the break caused by removal of RNA is distributed at random along the molecule. Hybridisation experiments with labelled DNA fragments which were made immediately after initiation of replication to the strands of Col E1 DNA treated successively with endonuclease *Eco*R1 and exonuclease III show that the relaxation break is located at the origin/terminus region.

³H-labelled relaxation complexes were converted to the open-circular forms as described in the legend to Fig. 1. After purification, they were treated with exonuclease III, which acts on the 3' end of double-stranded DNA¹¹. Two samples were prepared in which approximately 10 and 25% of the DNA was digested. After inactivation of the enzyme, the molecules were further treated with endonuclease *Eco*R1 to convert them to linear structures⁶. Electron microscopic examination⁶ of these molecules showed that they contained only one single-stranded region as indicated by the formation of a bush or a thinner stretch between thicker threads in molecules spread by the ammonium acetate technique or the formamide technique, respectively. This finding indicates that the molecule contains only a single break in one of the strands. Representative molecules spread by the ammonium acetate technique are shown in Fig. 1.

To determine the location of the relaxation break, the lengths of double-stranded regions on either side of the bush in each molecule were measured. The result presented in Fig. 2 shows that the length of the shorter double-stranded region was approximately 20% of the length of the untreated molecule independent of the extent of digestion but that of the longer double-stranded region was reduced as the digestion progressed. This indicates that the relaxation break is located approximately 20% of the length from an end of the molecule created by cleavage by endonuclease *Eco*R1 and the end has the 3' end of the H strand in which the relaxation break exists³. Since the origin/terminus region is located approximately 20% of the length from a molecular end formed by endonuclease *Eco*R1, the origin/terminus region and the relaxation break are closely located or they sit at symmetrical positions relative

to the *EcoRI* site. The following experiments were performed to distinguish between these two possibilities.

In a soluble system, Col E1 DNA replication proceeds through an early replicative intermediate which has a small replication loop containing a 6S DNA fragment(s)^{12,13}. Fragments of 6S DNA labelled with α -³²P-dTMP were purified and those hybridised to H strands of Col E1 DNA were isolated (legend to Table 1). The ³²P-labelled DNA was then annealed to the denatured DNAs prepared by the following procedure. First, closed-circular Col E1 DNA was cleaved by endonuclease *EcoRI* to a linear form. The linear DNA was then treated with exonuclease III to render 40% of the DNA acid-soluble. By this treatment approximately 40% of the lengths of both strands was assumed to be digested from their 3' ends because digestion by exonuclease III proceeds more or less synchronously from the 3' ends, as shown by Fig 2. The DNA treated with endonuclease *EcoRI* and that treated with endonuclease *EcoRI* and exonuclease III were denatured and fixed on membrane filters. They were annealed to a mixture of ³²P-labelled 6S DNA (hybridised to H strands and separated from them) and ³H-reference DNA. Table 1 shows that the exonuclease-III-treated DNA had a drastically reduced capacity to hybridise the 6S DNA while it had a capacity to hybridise to the reference DNA (prepared by

shearing and denaturation of the total Col E1 DNA) corresponding approximately to the extent of digestion. The result indicates that the 6S DNA was hybridised to H strands at a region within 40% of the molecular length from the 3' end created by endonuclease *EcoRI*. Together with the result presented in Fig 2, this proves that the relaxation break is at or near the region where replication is initiated. By its location at the origin/terminus region, the relaxation protein is probably involved directly in replication or its regulation. The absolute polarity of Col E1 DNA strands was determined by the above results as follows: the 3' end of the H strand of a linear molecule created by cleavage by endonuclease *EcoRI* is located closer to the origin/terminus region than the 5' end of the strand.

Table 1 Hybridisation of 6S DNA, prehybridised to H strands, to denatured Col E1 DNA previously treated with endonuclease *EcoRI* and exonuclease III

Denatured DNA fixed on a filter	Radioactivity in hybridised DNA (c.p.m.)	
	6S ³² P-DNA	Reference ³ H-DNA (sonicated and denatured)
DNA treated with <i>EcoRI</i> (a)	163 (59%)	13,000 (62%)
DNA treated with <i>EcoRI</i> and ExoIII (40% digestion) (c)	22 (8%)	6,830 (33%)
Input DNA (c.p.m.)	275 (100%)	20,750 (100%)

³H-labelled closed-circular DNA was prepared from A745 (Col E1) *thy* cells which had been treated with chloramphenicol (180 μ g ml⁻¹) for 15 h at 37°C in a medium containing ³H-thymine (1 mCi mmol⁻¹). Purified closed-circular DNA⁹ was treated with *EcoRI* to convert more than 95% of the molecules to linear forms⁶. The reaction was terminated by the addition of EDTA to 10 mM. The linear DNA was further treated in three different ways as follows: (a) the DNA was denatured by 0.1 N NaOH followed by neutralisation by 1 N HCl; (b) the DNA was denatured by heating in the presence of poly(U,G) and centrifuged in a CsCl density gradient⁹ and the DNA in the heavy peak was dialysed and self annealed as described for the preparation of separate strands of λ phage DNA¹⁴; (c) the DNA preparation was heated at 80°C for 10 min to inactivate *EcoRI* and diluted fivefold with 0.07 M Tris-HCl, pH 8.0, after addition of MgCl₂ to 7 mM, the DNA was treated with exonuclease III to render 40% of DNA acid-soluble and then denatured by 0.1 N NaOH followed by neutralisation. The DNAs were fixed on membrane filters (Schleicher & Schuell, Bac-T-Flex B6) and treated with the preincubation mixture¹⁵. The amount of DNA fixed on a filter was approximately 2 μ g for whole DNA (a), 2 μ g for H strands (b) and 1.2 μ g for exonuclease III treated DNA (c). ³²P-labelled 6S DNA was prepared *in vitro* in a standard reaction mixture containing an extract of YS10(Col E1) cells, α -³²P-dTTP (5 Ci mmol⁻¹), glycerol and spermidine as described previously¹³. Early replicative intermediates (26S) were isolated and labelled 6S DNA was released by heating at 90°C for 2 min¹². The DNA was then annealed with a filter with fixed H strands for 18 h at 64°C in an incubation mixture (1 ml)¹⁵. The filter was washed¹⁴ and then treated with 0.5 ml of 0.05 N NaOH at 37°C for 1 h. The filter was removed and the solution was neutralised by 1 N HCl. Approximately 90% of ³²P-DNA was recovered. The prehybridised ³²P-DNA was mixed with approximately 0.1 μ g of Col E1 DNA labelled with methyl-³H-thymidine (2 Ci mmol⁻¹). The ³H-DNA was previously sonicated for 5 min in a Raytheon sonicator, denatured by 0.1 N NaOH and then neutralised by 1 N HCl. The mixed DNA solution (1 ml) was distributed in vials containing denatured DNA fixed on filters as indicated, and the mixtures were incubated at 64°C for 18 h to permit annealing. Filters were washed and radioactivity was measured. ³H-radioactivity from DNA that was first fixed to the filter was subtracted from the radioactivity obtained. The subtracted count was less than 5% of the count observed.

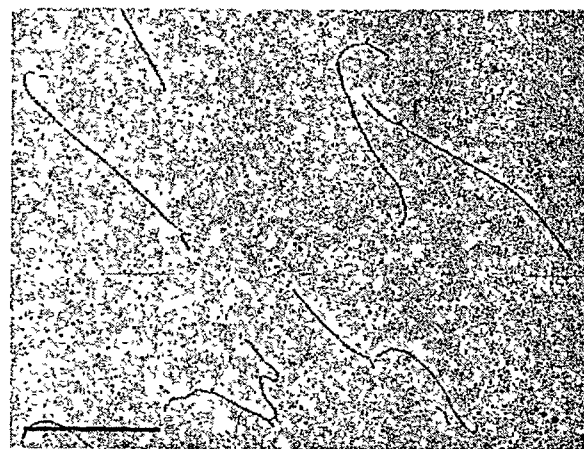


Fig 3 Electron micrograph of molecules containing RNA which had been treated successively with RNase A, exonuclease III and endonuclease *EcoRI*. Closed-circular molecules containing RNA were prepared⁴ from A745 (Col E1) *thy* cells which had been treated with chloramphenicol (180 μ g ml⁻¹) for 15 h at 37°C in a medium containing methyl-³H-thymidine (8 mCi mmol⁻¹). After treatment with RNase A (900 μ g ml⁻¹ Worthington, heated at 100°C for 5 min) at 37°C for 30 min, open-circular molecules were isolated. Approximately 10% of the total amount of the DNA was digested with exonuclease III and the molecules were cleaved by endonuclease *EcoRI*. They were examined under the electron microscope.

Closed-circular molecules which contained RNA as a result of their replication in the presence of chloramphenicol were examined next. Molecules were prepared from bacteria which had been incubated for 15 h in the presence of chloramphenicol⁴. By treating with RNase A (ref 4) approximately 50% of the molecules were converted to open-circular molecules which were isolated by sucrose density gradient centrifugation. After digestion of approximately 10% of the open-circular DNA by exonuclease III, which is known to act also on RNA hybridised to DNA¹⁶, it was converted to linear molecules by treating with endonuclease *EcoRI*. When these molecules were examined under the electron microscope, most of them had a single bush (Fig 3). The double-stranded regions of the linear molecules were measured and the length of the shorter double-stranded region is plotted against that of the longer one (Fig 4). Clearly the site of the RNA is distributed at random along the molecules. This conclusion was confirmed by another method. After RNase A treatment, the DNA was phosphorylated at the exposed 5' termini with γ -³²P-ATP and polynucleotide kinase¹⁷. They were treated with endonuclease *EcoRI* and subjected to agarose gel electrophoresis after heat denaturation. The ³²P radioactivity was equally distributed in molecules of various lengths (data not shown). These results eliminate the possibility that the RNA is involved in the initiation of the replication cycle of Col E1•DNA⁴ since replication is initiated in the fixed region⁶⁻⁸ even during

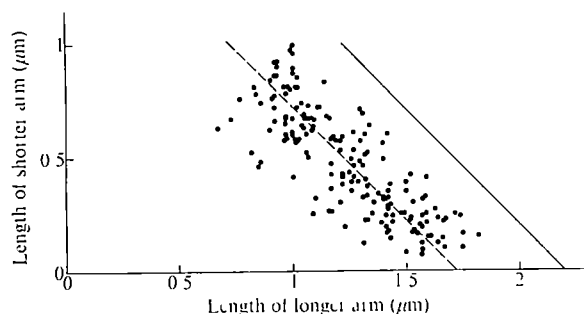


Fig 4 The position of the bush in each molecule in electron micrographs of the sample described in the legend to Fig 3. Each of the 175 points represents one molecule with a bush. Ten molecules with two bushes are not included. The sum of the lengths of both double-stranded regions at any point on the diagonal solid line represents the average length of untreated molecules ($2.22 \pm 0.09 \mu\text{m}$, 100%) and that on the diagonal dotted line shows the average length of the sum of double-stranded regions of a digested molecule ($1.72 \pm 0.15 \mu\text{m}$, 78%).

growth in the presence of chloramphenicol (J. Inselburg, personal communication). It is more likely that it is a remnant of RNA formed during chain elongation and is normally removed soon after its formation. Alternatively, it is still possible that the RNA is not formed during the normal process of DNA replication but resulted from the special conditions present during the chloramphenicol treatment.

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Regulation of expression of *lac* operon by a novel function essential for cell growth

THE study of the *in vitro* transcription of the *lac* operon using a soluble fraction of bacteria and DNA containing the genes of the *lac* operon, indicates that gene expression is controlled by both the inducer-operator interaction¹ and the action of

cyclic AMP²⁻⁶. We have studied the mutant *tsC42* (described previously⁷) and report that the *in vivo* expression of the *lac* operon is under the control of a phase-specific protein which is only synthesised or activated 20-30 min before cell division. This protein is essential for both the formation of membrane components and cell growth, as described previously⁷. We describe procedures for the isolation of the mutant and report its specific inability to express the *lac* operon at the transcriptional level.

The formation of cytochrome *b*₁ (ref. 8), L-α-glycerophosphate uptake systems⁸ and the β-galactoside transport systems (M. O., unpublished) occur at the same particular phase in the cell cycle of *Escherichia coli*. Furthermore, when the loss of viability of an unsaturated fatty acid auxotroph was followed after removal of the fatty acids, the cells died in a stepwise manner at a cell phase where the membrane components described above were formed (M. O., unpublished). This suggested that the corresponding processes involved in the formation of cytochrome *b*₁ and so on must be concerned with other important cellular events. We therefore selected the mutant with a phase-specific defect, assuming that cell growth is temperature-sensitive and the formation of the β-galactoside transport system is under the control of the corresponding *ts*-mutation.

A derivative of *E. coli* K12, strain AT713 was treated with the mutagen nitrosoguanidine as described previously⁹ and the cells were plated on λ-agar plate⁹, supplemented with 0.1% glucose after full segregation, and incubated at 30°C. When colonies developed to a diameter of 1.5-3 mm, the plates were transferred to 42°C. After 60 min incubation, the colonies were sprayed with a solution containing 4 mM isopropyl-β-D-thiogalactoside (IPTG) and further incubated for 60 min at the same temperature. Non-inducible colonies were detected by spraying with 4 mM o-nitrophenyl-β-D-galactoside (ONPG) solution containing chloramphenicol (2 mg ml⁻¹). Colonies showing less or no colour development were removed and

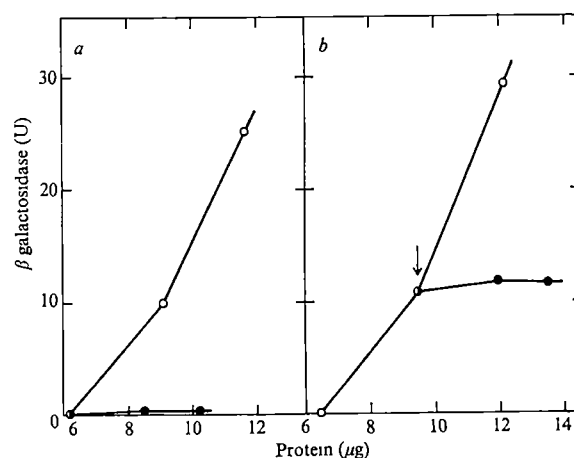


Fig 1 Formation of β-galactosidase in the mutant *tsC42*. Cells grown for several generations at 30°C in A medium¹⁰ were divided into two parts: one immediately transferred to 42°C (●), the other maintained at the same temperature (○). β-galactosidase was induced by the addition of 0.2 mM IPTG at the time of culture division (a) or 30 min before culture division (b). Extreme reductions in β-galactosidase formation were observed in both cases at the non-permissive temperature. In the culture was divided at the arrow. The β-galactosidase assay was performed according to a method of Pardee *et al.*¹¹. A unit corresponds to μmol ONPG hydrolysed per h per ml. Growth is expressed as increase in protein, measured according to the method of Lowry *et al.*¹². The activities of β-galactoside transport system induced by 0.2 mM IPTG at a permissive temperature were measured at 30°C and 42°C to show the intactness of the system. These activities and the ratio of 42°C/30°C are almost identical to those of the parental strain AT713, indicating that the reduced formation of β-galactosidase at a non-permissive temperature is not a result of a decrease in the transport of the inducer (Table 1).

clones, the growth of which is temperature-sensitive, were selected using the replica method on complete agar plates. Out of 105 non-inducible clones, four strains showed the expected phenotypes. *tsC42* was one of these.

The β -galactoside transport system was very poorly formed at a restrictive temperature in the mutant *tsC42* when assayed using an *in vivo* ONPG hydrolysis according to the method of Wilson *et al.*¹⁰ It was found later that the mutant also showed no β -galactosidase activity (Fig 1). The formation of the β -galactoside transport system was similarly arrested at a restrictive temperature (data not shown).

To find out whether the functional inability in the mutant *tsC42* occurred at the transcriptional or post-transcriptional level, we studied mRNA synthesis. DNA-RNA hybridisation experiments (Fig 2a), performed with $\phi 80$ *plac* DNA, indicate that the amount of the *lac*-specific mRNA is considerably reduced at 42° C (about 20% of that at 30° C), in spite of the fact that the total amount of pulse-labelled RNA is almost identical at both temperatures (data not shown). The increase in β -galactosidase activity at 42° C was measured in a parallel experiment and was about 20% of that at 30° C. Such similar values for the *lac*-mRNA and β galactosidase, suggest that the functional defect in the mutant *tsC42* lies at the transcriptional level. The *trp*-mRNA synthesis, detected by hybridisation with $\phi 80$ *ptrp*-ED-DNA, is shown in Fig 2b. The amounts of mRNA synthesised in the derepressed state at 30° C and 42° C are almost identical, in contrast to the *lac*-mRNA. Thus, *trp*-mRNA synthesis proceeds normally in a restrictive condition, transcription not usually being impaired but specific for the *lac* operon.

Cyclic AMP seems to be required for the expression of the *lac* operon¹⁴⁻¹⁷. We have therefore examined the effect of cyclic AMP (1 mM) on the formation of β galactosidase in the mutant *tsC42* and found no detectable stimulation at a restrictive temperature. Furthermore, this reagent could not restore the inability to grow at 42° C. Perlman and Pastan¹⁷ have reported that a mutant lacking adenyl cyclase is unable to grow on lactose, maltose, arabinose, manitol and glycerol in the absence of cyclic AMP but does grow on glucose, fructose and galactose. The growth inability of the mutant *tsC42* at 42° C thus examined was not affected by the sugars mentioned. These results indicate that the defect in the mutant is not associated with functions related to cyclic AMP metabolism. Cyclic AMP acts more generally on the synthesis of a number of inducible enzymes.

Spontaneous reversion frequencies to temperature-resistance were measured in two different clones on complete medium agar plates. Each clone showed the values of 4.6×10^{-7} and 10×10^{-7} , respectively, suggesting a single mutational defect. All twenty revertants so far examined regained the full inducibility of β galactosidase at 42° C. In three of these, the ability to form succinate dehydrogenase and L- α -glycerolphosphate dehydrogenase was completely restored at 42° C. This suggests that the loss of the expression of the *lac* operon is also associated with a defect in a function essential for cell growth and the formation of membrane components. The expression of the *lac*-operon may require a newly synthesised membrane component to effectively operate the protein synthesising machine or as a modulator of

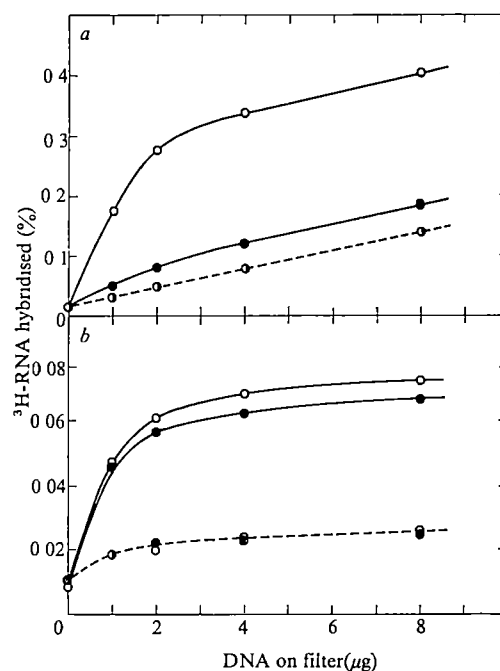


Fig 2 a, Exponentially growing cells cultured in medium A (ref 10) divided into four parts, two of them further incubated at the same temperature (30° C) and the others transferred to 42° C. Each group was incubated in the presence (induced) or absence (uninduced) of 0.2 mM IPTG, respectively. b, Exponentially growing cells cultured in Davis's minimal glucose medium with or without tryptophan (100 μ g ml⁻¹) were divided into two parts, one repressed and one derepressed cell were further incubated at 30° C and the others transferred to 42° C. Cells were labelled for 1 min by the addition of 100 μ Ci ³H-uridine (specific activity 26 Ci mmol⁻¹) at 20 min in the case of (a) and 15 min in the case of (b) after the start of the various conditions of incubation. The RNAs from (a) and (b) were extracted with hot phenol equilibrated with a sodium acetate buffer (pH 5.2) containing 0.5% SDS (ref 13) and hybridised with increasing amounts of $\phi 80$ *plac* DNA or $\phi 80$ *ptrp* DNA fixed on membrane filters according to the method previously described¹³. Ordinate, per cent (added) total radioactivity. ○—○, induced or derepressed at 30° C, ●—●, induced or derepressed at 42° C, ○---○, uninduced or repressed at 30° C, ●---●, uninduced or repressed at 42° C.

tertiary structure of DNA leading to an active form of the genes (Sankaran and Pogell¹⁸ suggested that the expression of the *lac* operon may be correlated with an alteration in the conformation of DNA).

Preliminary experiments in which cells were labelled with ¹⁴C-arginine at 30° C and with ³H-arginine at 42° C to distinguish the membrane components formed at a permissive temperature from newly-formed ones at a non-permissive temperature showed that the incorporation of ³H-arginine was restricted to several membrane components separated by SDS polyacrylamide gel electrophoresis.

In vitro studies have revealed that *lac*-DNA, RNA polymerase, cyclic AMP receptor protein, cyclic AMP and *lac*-repressor and inducer are the elements controlling *lac* transcription. The mutational defect in *tsC42* is distinct from those in that the defect is phase-specific, the corresponding function is only operative 20–30 min before cell division and is essential for cell growth and membrane synthesis.

We thank Dr H. Mitsui for discussions, Drs Ozeki and Ohshima for $\phi 80$ *plac* phage, and Drs Imamoto and Segawa for $\phi 80$ and $\phi 80$ *ptrp*-ED-DNA. This work was supported in part by the research Fund of the Ministry of Education of Japan.

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Table 1 Activity of ONPG transport in the mutant *tsC42* and the parent AT713

	ONPG hydrolysed 30° C (μ mol h ⁻¹ ml ⁻¹)	<i>in vivo</i> at 42° C	Relative activity 42° C/30° C
<i>tsC42</i>	0.72	1.74	2.4
AT713	0.81	2.43	3.0

Cells grown in A medium¹⁰ for several generations at 30° C were incubated with 0.2 mM IPTG for 30 min at the same temperature and β -galactoside transport was induced. Cells once washed with A medium¹⁰, containing chloramphenicol (40 μ g ml⁻¹) but without casamino acids, were suspended in the same medium. Transport was measured according to the method of Wilson *et al.*¹⁰

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C_H3 domain of IgG as binding site to Fc receptor on mouse lymphocytes

MEMBRANE receptors recognising the Fc portion of immunoglobulin molecules (Fc receptors) are found in many cells of the immune system¹⁻⁴. Fc receptors on lymphocytes are readily detected by a rosette test^{3,5,6} and this reaction is inhibited by pretreatment of the lymphocytes with IgG (ref 3). IgG proteins lacking almost the entire C_H1 and C_H3 homology regions have been obtained from mutant cell lines of MOPC 21, a plasmacytoma secreting IgG1 (refs 7 and 8) and the extent of the deletions determined (Fig 1). To identify that part of the IgG molecule which interacts with the Fc receptor, we have tested the ability of these IgG proteins to inhibit Fc rosette formation on murine lymph node cells. We show (Table 1) that an intact C_H3 region is essential for the binding of IgG to Fc receptors on lymph node cells.

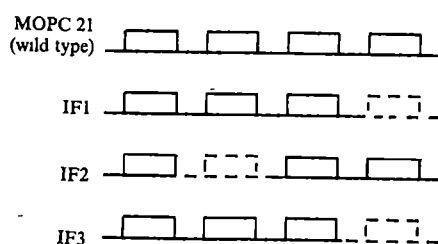


Fig. 1 Structure of the mutant proteins. Dotted lines indicate deletions in the heavy chains, shown diagrammatically, with the four intra-chain disulphide bridges. IF1, IF2 and IF3 were cloned tissue culture lines of the mouse plasmacytoma MOPC 21. IF2 has a deletion in the heavy chain of residues 121 to 214 corresponding to the wild type protein^{8,10}, that is, of almost the entire C_H1 region. IF1 has a deletion of residue 358 onwards¹⁶ and IF3 of residue 342 onwards (K. A. and C. Milstein, unpublished). The secreted protein used in the experiments was purified from the serum of mice bearing the respective tumours, using ammonium sulphate precipitation and DEAE cellulose chromatography. The purity was checked using electrophoresis on cellulose acetate strips, and isoelectric focusing on polyacrylamide gels.

The ability of IF2 protein to inhibit Fc rosette formation shows that the C_H1 region is not the site through which IgG binds to Fc receptors. The failure of IF1 and IF3 proteins to inhibit Fc rosettes shows that an intact C_H3 region is required for the binding of IgG to Fc receptors. Since both IF1 and IF3 possess intact C_H2 regions, this region may not be required for binding to Fc receptors. It is possible, however, that the absence of the C_H3 region in IF1 and IF3 can result in a rearrangement of the C_H2 region, destroying an active site wholly or partly within this region. The inability to detect the C_H3 region of membrane-bound IgG on lymphocytes, with a fluorescent antiglobulin possessing an anti-C_H3 region

Table 1 Inhibition of rosette formation with IgG proteins

Inhibitor	Percentage reacting cells
Experiment 1	± s.e.
No inhibitor	33.8 ± 2.3
MOPC 21 (parent IgG) 0.58 mg ml ⁻¹	18.0 ± 2.9
IF1 (C _H 3 deletion) 0.58 mg ml ⁻¹	30.9 ± 1.9
IF2 (C _H 1 deletion) 0.58 mg ml ⁻¹	3.3 ± 3.7
Experiment 2	
No inhibitor	34.1 ± 2.5
MOPC 21 (parent IgG) 10 mg ml ⁻¹	0.1 ± 0.1
IF1 (C _H 3 deletion) 10 mg ml ⁻¹	35.9 ± 1.1
Experiment 3	
No inhibitor	43.6
MOPC 21 (parent IgG) 5 mg ml ⁻¹	9.4
IF3 (C _H 3 deletion) 5 mg ml ⁻¹	41.4 ± 1.8
IF1 (C _H 3 deletion) 5 mg ml ⁻¹ (heat aggregated)	43.8 ± 2.9

Pooled lymph node cells from BALB/c mice were used in the experiments. Fc rosette formation was carried out as previously described^{5,6}. For inhibition of Fc rosettes, lymphocytes were pretreated with inhibitor protein at the concentrations stated for 15 min at 0°C before rosette formation. For one experiment IF1 protein was heat-aggregated at 63°C. The results are the means of more than three replicate preparations when the standard error of the mean is given. Two observations in experiment 3 were the results of counting over 200 cells in a single preparation.

specificity, also suggests that it is the C_H3 region which interacts with the lymphocyte membrane⁹.

In an extension of the domain hypothesis, Edelman *et al*¹⁰ proposed that each of the homology regions of the polypeptide chains of immunoglobulin molecules folds to form a compact domain which has evolved to perform independent functions. Thus the V_H and C_H2 regions of IgG possess the antigen binding and complement fixing sites¹¹, respectively. The binding of IgG to human monocytes¹² and guinea pig macrophages¹³, possibly through a site in the C_H3 region, has been suggested as evidence for the domain hypothesis. The binding of IgG to Fc receptors on K cells¹⁴, neutrophils¹⁴ and a non-secreting plasmacytoma¹⁵ also requires an intact C_H3 region. The C_H3 region of IgG seems therefore to possess the property of binding IgG to Fc receptors, which may be functionally relevant, on many types of cells.

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matters arising

Orogenic zones in central Australia

DUFF and Langworthy¹ present a number of arguments purporting to confound any suggestion of a subduction model² for the Giles Complex–Woodroffe Thrust Zone located along the contact of the Amadeus Basin with the Musgrave Block. During the orogeny associated with this contact, the Petermann Range orogeny³, the north-recumbent nappes developed and thrusting occurred. I have confined my arguments in all instances to this orogenic belt. Any later orogeny, for example, the Alice Springs orogeny, affecting other regions may well be explained otherwise.

this age that is correlated with the tectonic–orogenic episode. If the Petermann Range orogeny is some 1,100–1,150 Myr old, the Arunta and Musgrave Blocks would have moved as a unit from this time onwards, in keeping with the palaeomagnetic evidence.

Duff and Langworthy list features that they claim do not occur in the Petermann Range orogenic belt, thus disproving a subduction model for this region. In this respect, note that acid intrusives, for instance, occur within the orogenic belt⁴. It has been argued elsewhere that the Giles Complex may well be segments of an ophiolite sequence and it has reasonably been suggested that the extent of represen-

The presence of possible examples of ensialic mobile belts within African and North American cratonic regions⁵ does not preclude the possibility of other orogenic types in specific locations within the Australian craton. It seems to me that a subduction model more than reasonably fits the data available in these areas for the Petermann Range orogeny. Present-day intraplate tectonic compression⁶ does not support the argument for ensialic orogeny unless a correlation can be shown between regions of intraplate compression and regions of thick sedimentation of a specific nature and with igneous activity. This constraint is met by regions of present-day subduction and continental shelf regions and thus these zones can be used as models for past geological events.

In conclusion, in the case of the Petermann Range orogeny the ensialic concept does not explain the following features: the major lithological and structural differences of the basement rocks in contact along the thrust zone, the mafic/ultramafic zone; the significance and emplacement of the Giles Complex; the asymmetric nature of nappe distribution and the recumbency of these structures to the north; the spatial and temporal distribution of discrete tectonic elements from south to north. It also seems that the most critical data in the future will be palaeomagnetic and geochronological data relating to the Arunta and Musgrave Blocks specifically.

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Table 1 Radiometric ages for the Petermann Ranges and for an intrusion in the Musgrave Block

Rock types	Age (Myr)	Method*
Granitic gneiss	1,190 (whole rock)	Rb/Sr (3)
	600 (biotite)	
Biotite granite	1,150 (whole rock)	Rb/Sr (3)
	600 (biotite)	
Adamellite	1,123 (whole rock)	Rb/Sr (5)
	1,094 recalculated	
	1,077–1,092	K/Ar (6)

*Numbers in parentheses indicate source reference no.

A fundamental argument presented by Duff and Langworthy relates the time of the orogeny to palaeomagnetic evidence indicating that the Arunta and Musgrave Blocks have moved as a single unit since the late Proterozoic or early Palaeozoic. Even if the palaeomagnetic data⁴ are accepted, a subduction model is in no way contravened, as geochronological evidence clearly indicates that the Petermann Range orogeny occurred about 1,110–1,150 Myr ago. Duff and Langworthy have quoted Forman³ as specifying an age of 600 Myr for this orogeny. Forman gives radiometric ages from specimens collected from the Petermann Ranges and these figures, together with an age given by Wilson and Green⁵ for an adamellite intrusion in the Musgrave Block, are shown in Table 1. The granitic rocks show intrusive contacts with folded quartzites³ in the Petermann Range area.

The question then is whether the whole rock age or the biotite age is taken as dating the orogenic episode. Most authorities now regard biotite ages as excessively low because of the low blocking temperature of this mineral⁷. The biotite age is probably indicative of a late stage of cooling of the crystalline rock and this could be related to uplift and erosion. The whole rock age is generally accepted as the age of initial crystallisation, so it is

tation of certain criteria indicative of subduction, for example, blue-schist facies rocks and calc-alkaline volcanics is very much dependent on the depth of erosion². Erosion would be considerable in Precambrian orogenic belts. Rocks outcropping in the Petermann Range orogenic belt have formed under high pressures and temperatures, that is, at deep crustal levels, so low-temperature and low-pressure rocks are unlikely to be found *in situ*, but this does not mean that such rocks did not form.

Matters arising

Matters Arising is meant as a vehicle for comment and discussion about papers that appear in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted. Neither contribution nor reply (if one is necessary) should be longer than 300 words and the briefest of replies, to the effect that a point is taken, should be considered.

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DRS DUFF AND LANGWORTHY REPLY—Davidson^{1,2} believes that a simple plate-subduction model explains certain features of the Musgrave Block, adducing support solely from the alleged similarity of sections through the central Australian shield with 'cartoon' plate-tectonic sections³. He argues^{1,2} that the usual features of plate-edge tectonism—pillow basalts, pelagic sediments, calc-alkali volcanics,

blue schists—are absent because they are above the depth of present erosion. Since that proposition can be neither demonstrated nor refuted his essential thesis amounts to a non-argument. Moreover, the notion of supracrustal erosion of subduction evidence² is difficult to reconcile with the proposed preservation of segments of the ophiolite suite.

He also envisages² a single 1,150 Myr tectonic event, the Petermann Ranges orogeny⁶, during which thrusting, igneous activity and folding, affected basement and cover south of the Amadeus Basin. Within this event, he incorporates the generation of the Woodroffe Thrust as well as the Petermann Ranges nappes in the Dean Quartzite. The whole-rock age of 1,150 Myr used by Davidson² to date the orogeny, however, applies only to a granite unconformably underlying the deformed Dean Quartzite^{4,5}; and it has not been demonstrated that basement thrusting further south was necessarily synchronous with cover deformation or granite intrusion. The Petermann Ranges orogeny therefore probably occurred about 600 Myr ago⁶. As we have indicated⁷, available palaeomagnetic data⁸⁻¹⁰ preclude relative movement between blocks during this period.

The Giles Complex is not, as claimed^{1,2}, a 'segment of an ophiolite suite'. The complex consists of intrusive sheets that contain relatively high pressure assemblages¹¹ suggesting that it was intruded into the lower continental crust¹¹⁻¹³. The Gosses Pile^{1,13} is a layered, ultramafic intrusive sheet that crops out 50 km south of the Woodroffe Thrust and consists of 'orthopyroxenite, websterite, and olivine-orthopyroxenite arranged in a cyclic sequence such as is found in the ultramafic parts of other layered intrusions'¹³. In no way can the Giles Complex be construed as segments of a Proterozoic oceanic crust.

As Davidson¹ himself has previously noted there are no major lithological and structural differences of basement rocks across the Woodroffe Thrust zone.

Geological¹⁴ and recent palaeomagnetic¹⁵ work in certain Precambrian regions has indicated that uniform application of plate-subduction models to such terrains is suspect¹⁵, and we believe that indiscriminant application of such concepts to terrains not fully understood, may serve only to mask the actual tectonic processes.

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Lunar magnetic anomalies and the Cayley Formation

MAGNETIC anomalies may or may not relate to geological units. Strangway *et al.*¹ incorporated geological data in attempting to equate surface anomalies at the Apollo 16 site with those recorded from orbiting Apollo subsatellites, and then correlated the northern plains in the crater Van de Graaff with the Cayley Formation. This correlation is incorrect for three reasons.

First, planar fill in the north end of Van de Graaff is low-albedo mare material with mare ridges, not Cayley-like plains (Fig. 1). Also, the magnetic anomaly near Van de Graaff lies between craters Aitken and Van de Graaff², so that the anomaly could just as easily be ascribed to the acknowledged maria^{3,4} in Aitken. Finally, Strangway *et al.*¹ cited similar crater densities for the Van de Graaff mare and the Cayley Formation at Descartes, thus implying similar ages. Apollo mapping camera photographs show, however, that the maria in Aitken and Van de Graaff (NASA photographs AS17-1385 and AS15-0076) have similar primary crater densities (Fig. 1)

whereas the Cayley plains seem to have significantly more 0.5-1.0 km craters (NASA photograph AS16-0162) and are therefore older.

Orbital magnetic data do not substantiate the correlation of Cayley-like plains with any particular magnetic signature. The other significant magnetic anomalies on the centre far side are not near or over large areas of light plains but instead occur over a wide variety of terrain²; there is no significant anomaly over Cayley Formation plains on the near side of the Moon. If flows of hot breccia from basins were the main cause of lunar magnetic anomalies¹, then the basin Hertzprung should show a large anomaly because it is extensively filled with Orientale ejecta. Early reduction of Apollo 15 data² showed a weak anomaly, but this vanished after Apollo 15 and 16 data were integrated (L. R. Sharp and P. J. Coleman, unpublished data).

The outstanding magnetic anomaly between Aitken and Van de Graaff (Fig. 1) correlates with no unique geological feature. The same type of geology occurs elsewhere without magnetic signatures. Therefore, the best explanation for the anomaly remains a subsurface source².

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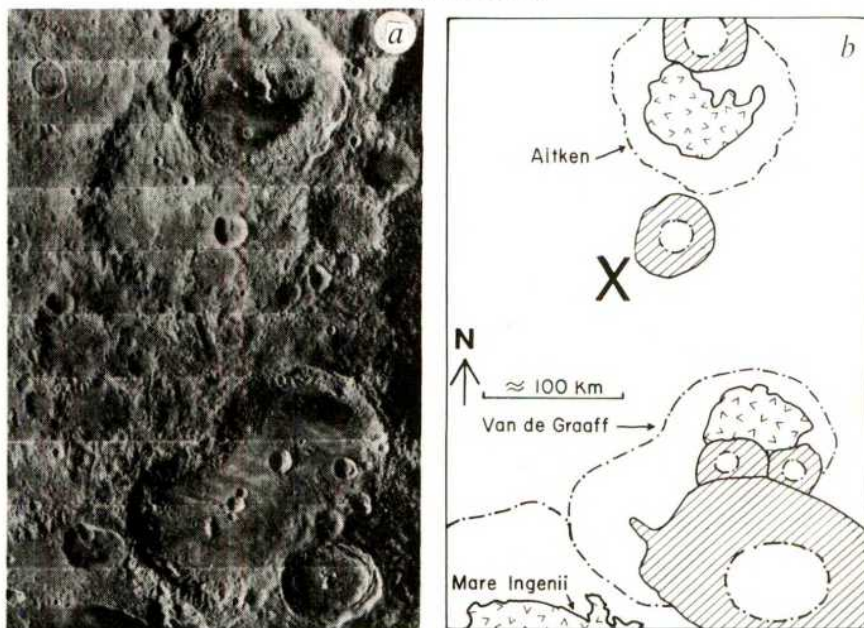
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Fig. 1 *a*, NASA photograph of the Aitken-Van de Graaff area, showing the low-albedo maria in these two craters and in Mare Ingenii (Orbiter II, moderate 33); *b*, sketch map shows approximate centre of the magnetic anomaly (X). —, Contacts; ---, crater rim crests; lined pattern, post-mare crater ejecta; 'v' pattern, mare rocks.



Shadow of the head of Comet Kohoutek

HOPKINSON, Elsworth, and James¹ have reported the detection of a straight dark streak running away from the head of Comet Kohoutek (1973f) for a distance of about 2.5° . They conjectured that the streak could be the shadow of the comet's head as seen in the dust tail of the comet. Their observations were made on January 9, 10, 11, and 12, 1974 at about 2200 UT.

We obtained photographic observations of Comet Kohoutek at the Joint Observatory for Cometary Research (JOCR) near Socorro, New Mexico. We have observations which overlap in time with those obtained by Hopkinson, Elsworth, and James; a sample taken on January 11, 1974 UT is shown (Fig. 1). Other JOCR plates have been discussed by Hyder *et al.*². Our primary plate emulsion was Ila-O, but we also have plates with Ila-E and Ila-F emulsions. Colour photographs (Ektacolor-L) were also taken.

We feel that the shadow, if real, should be found on our plates, but we have found nothing that we would interpret as the shadow of the head extending 2.5° (5.4×10^6 km) in the antisolar direction. At the time of the exposure shown in Fig. 1, the prolonged radius vector was very close to the axis of the plasma tail as projected on the plane of the sky. We have found two features which conceivably could be responsible for the earlier observations¹. First, a linear dark area was found on a Ila-E plate taken shortly before the Ila-O plate shown in Fig. 1. This dark streak is in the head, on the side away from the dust tail. Unfortunately, it is neither long enough nor in the correct position to concur with the observations of Hopkinson, Elsworth, and James. In addition, inspection of the Ila-O plate shows clearly that this dark area is just the space between tail rays of the plasma tail. Similar dark streaks pointing toward the nucleus are found on the other side of the head (Fig. 1), but they are not as prominent because the

scattered solar radiation from the dust tail tends to fill in the dark spaces. Second, on low resolution photographs the space between the plasma and dust tails could give the appearance of a dark streak.

Whatever the ultimate explanation, we note that our plate scale is $300'' \text{ mm}^{-1}$, and we estimate that our resolution (which we consider to be caused by plate scale, image quality and emulsion characteristics) is approximately 10 times greater. The difference in resolution is probably the root of the discrepancy.

Finally, in terms of cometary physics, we believe that observation of the shadow is unlikely for two reasons. First, our colour photographs indicate a low dust content and a relatively weak dust tail as compared with Comet Bennett (1961i). Thus, it is unlikely that observable dust would extend out to 5.4×10^6 km (2.5°) except near the axis of the dust tail. Second, the dust tail (presumably composed of micron sized dust) curves away from the antisolar direction. At 5.4×10^6 km from the nucleus, the axis of the dust tail lies well away from the antisolar direction.

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¹ Hopkinson, G. R., Elsworth, Y., and James, J. F., *Nature*, **249**, 233 (1974).

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Platelet monoamine oxidase in schizophrenics

MURPHY and Wyatt reported reduced monoamine oxidase (MAO) activity in blood platelets from 33 chronic schizophrenic patients¹ and 13 monozygotic twin pairs² compared with normal controls. They suggested that reduced platelet MAO activity may provide a genetic marker for vulnerability to schizophrenia. We predicted lower MAO activity might sensitise the

schizophrenic patient to the induction of symptoms by tricyclic antidepressants³.

We have completed a study of 24 anergic, depressed schizophrenic outpatients. Patients were free of psychoactive medication for 2 weeks and then blindly treated with either chlorpromazine (Thorazine, SKF) 100–1,200 mg daily and imipramine (Tofranil-Geigy) 150 mg daily or thiothixene (Navane-Roerig) 5–60 mg daily and placebo. Platelet MAO activity was measured following the drug-free period and weekly for 4 weeks on medication, using the method of Wyatt and Murphy^{1,2} with ¹⁴C tryptamine (1.6×10^{-5} M final concentration) as substrate. Blood samples from eight alcoholic patients and seven staff volunteers served as controls.

We found no differences in platelet MAO activity in the schizophrenic patients compared with alcoholics and volunteers. On the basis of MAO platelet activity (mean \pm standard deviation in nmol product formed per h per mg protein) individuals from all three groups could be assigned to either a low (1.62 ± 0.34) or high (3.09 ± 0.53) MAO group, with no overlap. Our low MAO group contained 13 schizophrenic patients, four alcoholics and three staff. The high MAO group consisted of 11 patients, four alcoholics and four staff. Discriminant function analysis distinguished high from low MAO level patients on the basis of hyperactivity (Katz adjustment scale factor), sleep disturbance and apathy (Hamilton depression scale factor). No significant differences in primary schizophrenic symptoms existed between patients in the low and high groups at baseline. Preliminary data analysis of 4-week improvement indicates low MAO group patients may do significantly less well on the chlorpromazine-imipramine combination. These findings are under investigation in schizophrenic inpatients.

We have been unable to replicate the reduced blood platelet MAO findings in schizophrenic patients as reported in *Nature* by Wyatt and Murphy¹. Individuals have either low or high MAO activity independent of diagnosis. Activity remains stable over at least five week periods. Platelet MAO activity may influence the schizophrenic patient's response to tricyclic medication.

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DRS MURPHY AND WYATT REPLY—Shaskan and Becker's interesting data may be clarified by other recent MAO

Fig. 1 Comet Kohoutek (1973f) on January 11, 1974 UT. This 2 min exposure was taken between 2:59:20 and 3:01:20 UT. The field shown is approximately $3^\circ \times 4^\circ$.



studies. In addition to our reports^{1,2}, Nies *et al.*⁴ observed significantly reduced MAO activity in schizophrenics, as did Meltzer and Stahl⁵, who also noted differences in substrate specificity differentiating chronic from acute patients. Our recent study of acute schizophrenics revealed normal MAO activity⁶, while a replication study of chronic schizophrenics again documented reduced MAO activity unexplained by differences in thyroid or sex steroid hormones, iron metabolism or drug treatment (in preparation). This distinction between chronic and acute schizophrenics is interesting in view of studies of adopted-away children of schizophrenics indicating that chronic and acute schizophrenia may be genetically distinct disorders⁷.

While the non-overlapping low-high distribution of MAO activities observed by Shaskan and Becker in 24 schizophrenics might represent a split between chronic and acute patients, the bimodal distribution in eight alcoholic and seven staff controls suggests the possibility of sampling inequities or technical differences in this very small sample. A typical unimodal distribution pattern for platelet MAO was observed in 167 normals⁸. Whether alcoholics should be included as 'controls' is problematic, as alcoholism has been linked to affective disorders, and patients with bipolar affective disorder have reduced MAO activity⁹.

We have also found platelet MAO activity to be a generally stable characteristic of individuals. We continue to view MAO differences as possibly related to vulnerability issues (which might, for example, result in chronicity) rather than to overt psychiatric disorders themselves^{2,8}. Shaskan and Becker's observation that low MAO patients may be relatively treatment-resistant is congruent with this concept. In studying non-hospitalised, drug-responsive patients, however, Shaskan and Becker have not replicated the conditions of our first study of chronic schizophrenics, but instead may have accomplished a combination study of schizophrenic subgroups. We look forward to further examination by them and others of the relationships between MAO activity, behaviour and behavioural disorders.

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Chloride ions cancel out inhibition of β -galactosidase activity by acid mucopolysaccharides

A SERIES of short reports on the effect of glycosaminoglycans on β galactosidase have been published¹⁻³. Kint³ has shown that cetylpyridinium chloride or albumin can partially overcome the inhibition of β galactosidase by the glycosaminoglycans accumulated in autopsy liver homogenates from mucopolysaccharidoses patients. We report that this inhibition is completely cancelled out by a pure physiological agent, that is, chloride ions, required for enzyme activity⁴⁻⁶.

The effect of various glycosaminoglycans and synthetic sulphated polymers on acid β -galactosidase activity is summarised in Table 1, which shows that hyaluronic acid was not inhibitory whereas all the sulphated polymers were inhibitory to varying extents. Chondroitin sulphate A and keratan sulphate were only mildly inhibitory but the synthetic sulphates were very strongly so. Chloride ions cancelled out all inhibition completely. The lowest concentration of chloride ions which prevented inhibition resulting from 83 $\mu\text{g ml}^{-1}$ heparitin sulphate was about 1.6 meq.

Other salient observations were as follows. (1) Chloride ions prevented inhibition if added simultaneously with inhibitor in the incubation mixture. Preincubation of enzyme with inhibitor at 37° C led to irreversible inactivation after 5–10 min and subsequent addition of chloride ions did not restore enzyme activity. (2) Inactivation of enzyme by glycosaminoglycans required heat and acidic pH (below 4.5). A mixture of enzyme and inhibitor maintained at 0° C did not result in inactivation or complex formation. The same mixture

Table 1 Effect of glycosaminoglycans on acid β galactosidase activity in the presence or absence of chloride ions

Addition	Enzyme activity (%)	
	–Chloride	+Chloride
None	100	138
Hyaluronic acid	116	139
Chondroitin sulphate A	73	135
Keratan sulphate	73	134
Dermatan sulphate	51	126
Heparitin sulphate	20	133
Dextran sulphate	7	88
Polyvinyl sulphate	5	80

A 15% homogenate of liver in distilled water was diluted 1:20 with water or 0.2 M NaCl and assayed for β galactosidase activity using 4-methylumbelliferyl- β -galactoside as substrate⁶. Incubations were carried out for 15 min at 37° C. The final concentration of chloride ions in the assay mixture was 33.3 meq., that of glycosaminoglycans/sulphated polymers 83 $\mu\text{g ml}^{-1}$.

Table 2 Effect of chloride ions on β -galactosidase activity in mucopolysaccharidoses patients and in controls

Liver homogenates	Enzyme activity	
	–Chloride	+Chloride
Control (1)	160	180
Control (2)	264	309
Hurler (MP-I)	7	22
Hunter (MP-II)	5	30
Sanfilippo (MP-III)	15	58

Conditions are as in Table 1. Enzyme activity is expressed as nmol substrate hydrolysed per h per mg protein.

on starch gel electrophoresis at 4° C (ref. 6) gave an isoenzyme pattern indistinguishable from controls.

The effect of chloride ions on β -galactosidase activity in liver homogenates from mucopolysaccharidoses patients and from controls is shown in Table 2. The results indicate that the apparent restoration in enzyme activity in the mucopolysaccharidoses patients was incomplete, suggesting that irreversible enzyme inactivation had occurred. Further inactivation of enzyme was prevented, however, by the presence of chloride during incubation, as evidenced by the three to fourfold increase in activity in all patients when chloride was included in the assay mixture.

It is now clearly established that the β -galactosidase abnormality in the mucopolysaccharidoses patients⁷ is not the primary genetic defect¹⁻³. The casual relationship between the reduction/inhibition of β -galactosidase activity and the clinical manifestations of the diseases is not known. Therapy along the lines of 'restoration' of β -galactosidase activity should be considered only when the above correlation is clearly demonstrated. If such therapy is required, chloride ions (administered for example as a saline infusion) should be chosen as they are relatively innocuous compared to the alternatives suggested⁸.

This work was done in the laboratory of Professor John S. O'Brien, Department of Neurosciences, University of California, San Diego. We thank Professor O'Brien for liver autopsy samples.

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reviews

THIS is an important book*. It is important because in it a man most competent to do so, sets out society's most important problem: self-control and regulation. It is a problem which has become increasingly urgent over the last decade. In this book Stafford Beer, perhaps the world's leading exponent of cybernetics in action, sets out the problem again and again in a series of addresses which he has given over the years: a presidential address to the Operational Research Society; a chairman's address to the International Cybernetics Congress; a presentation to the committee on Science and Astronautics of the US Congress; a keynote address to the Conference on Ecological Systems; a presidential address to the Society for General Systems Research; and many others. In each address the message is repeated, amplified and extended. Because the talks were given to a variety of audiences and because Stafford Beer writes in a readable style they can be understood by the non-specialist. Far from being boring the repetition serves to drive home the message just as walking round and round a building gives a lasting impression. The separate addresses are welded into a whole by a thesis running through the book (blue pages) and linked by a personal narrative (yellow pages) and a metasytemic overview (gold pages). On these other than white pages the script is broken down into what seems to be blank verse but what is really a grouping of words and phrases to make them more noticeable. This novel method is certainly effective even though it is irritating at times. It gives the effect of shouting, but that may only be until we get used to it.

Novel as they are the mechanics of the book and indeed the powerful highly personalised style of the author

are less important than the message. Stated simply the message is as follows: man must learn to control the increasing complexity of his world; there is a way of doing this known as cybernetics and general system theory; man's thinking and institutions make it impossible for him to adopt this method; result: loss of control and disaster.

"Thanks to the growth of complexity, which is very much a function of the

native courses ahead. The first is that nothing is done: we carry on as we are. Then the component of future trajectories that is in principle adaptive will, I predict, fail to adapt—because it is frustrated by an organisational straitjacket. And the component that is inexorable will, I predict, take society on to structural collapse or to its overthrow by revolution. The second alternative is that we should create a meta-system to handle the metathreat."

So the problem is clear: increasing complexity. And the solution is clear: the applied science of control and regulation tooled by computers. What then is the problem? Quite simply that the solution cannot be applied. That is really what the book is about. A plea, even an evangelical plea, for society to use the solution available to it. What is the alternative system that society seems to prefer? It is the classic 'muddle-through', a sort of creeping evolution that consists of doing nothing structural but reacting to the immediate situation and relying on cataclysmic upheavals for structural change. As Stafford Beer points out, society is so complex that reacting only to the immediate is quite ineffective and also overlooks the different lag and lead times in the system (witness our economic nonsenses). Furthermore, cataclysmic upheaval is likely to be catastrophic. Finally, the wrong decisions (as in nuclear affairs)

can lead to irreversible situations.

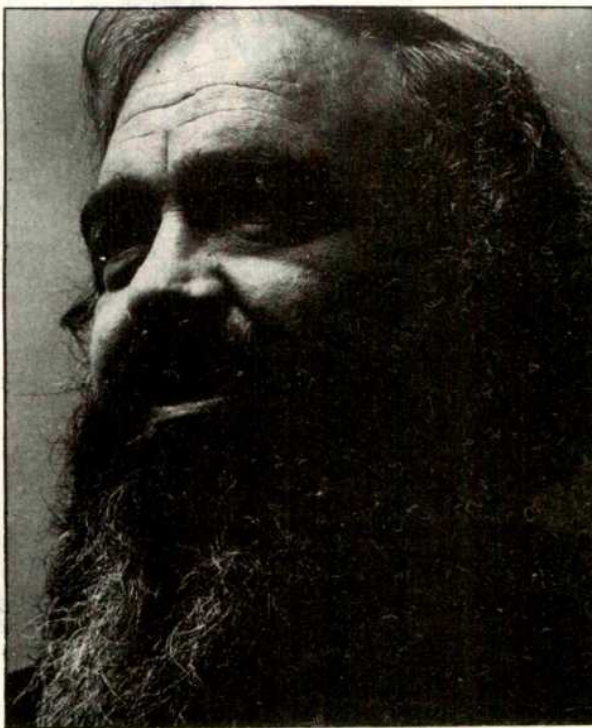
Why, then, is society so reluctant to adopt the system approach so clearly set out for it by Stafford Beer and his peers? The author thinks that we need a new kind of man: man the controller rather than man the maker.

"The difficulty is how to replace *Homo faber* with a new kind of man. He will not be man the maker any longer. He will be man the steersman—of large complex, interactive systems. I call him *Homo gubernator*."

Stafford Beer blames also our antiquated thinking habits and especially their stultifying dominance by the

New order for society

Edward de Bono



Stafford Beer

growth in data handling capacity and of the information explosion, society has outgrown the dynamic regulating capacity of its own hallowed structure."

"Handling complexity seems to be the major problem of the age, in the way that handling material substance offered challenge to our forefathers. Computers are the tools we have to use and their effective use must be directed by a science competent to handle the organisation of large, complex, probabilistic systems. This is the science of cybernetics, the science of communication and control."

"There seem to be only two alter-

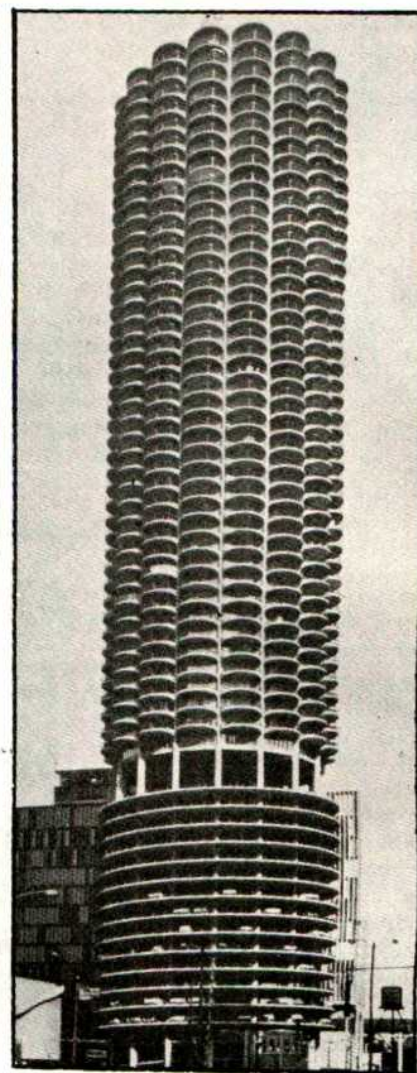
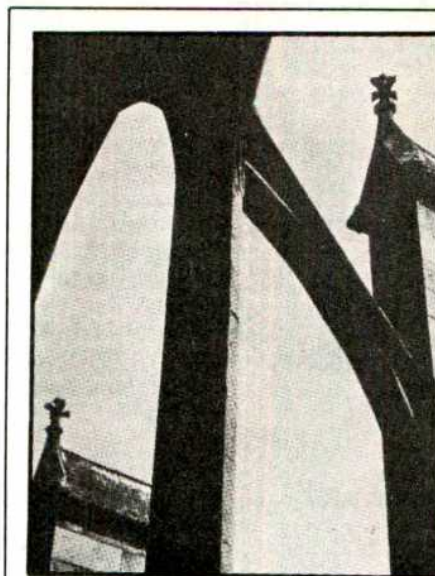
**Platform for Change*. By Stafford Beer. (Wiley: London and New York, January 1975.) £5.00.

Greek tradition of analysis. He points out that the Greeks had no concept of system: "As far as I can tell, the Greeks—even the greatest of the Greeks, Aristotle—had not the faintest glimmer of understanding here." I am very much inclined to agree with him. We forget just how much our academic and thinking traditions are dominated by the particulate analysis and logical reconstructions we have inherited from the Greeks. Our ordinary government is based entirely on such idioms: analyse, define, judge, choose, act. In one of the sections the author lets fly at the media in general and the BBC in particular for castrating any attempt by science to put over system concepts or science in general. This is a little unfair but there is no doubt that the media allocation to fundamental concepts such as those outlined by the author is infinitesimal (on the basis that no one would be interested). "... a colossal effort of will is required to dislodge the trammels of thought which have by now set in concrete."

Stafford Beer makes great use of the concept of metalanguage and meta-systems. A metalanguage is a language equipped to decide what is otherwise undecidable in lower order languages, and a metasystem does likewise: "In short I am making a straight appeal to the logical transcendence at the meta-systemic level of limitations imposed on the lower-order logics by Gödel's Theorem". At one point he attributes much of the disorder in society to the loss of the metasystems imposed by religious, moral and legal frameworks. He also tries to revive the idea of 'eudemony' (based on one of the Greek words for happiness) roughly meaning a "complicated image of well-being", suggesting it as the new measuring quality, or metric, of human systems.

The message is well put together and utterly convincing. Opponents will, of course, ask why those occasions when system planning was given its head were not great instances of its effectiveness. It is said the Vietnam war was organised on this basis. Stafford Beer himself was a chief adviser to Allende's Chile. The explanation offered by the author is that in no case were the systems able to operate effectively. Chile, he claims, was the victim of an international assassination. All that is true but should a system not be designed to take account of the environment in which it is supposed to behave?

We come now, I think, to the weakness of the argument. Systems can indeed optimise the control and performance of the units or concepts embodied in them. But what are those concepts to be? We can optimise the flow of 10-ton trucks along a road or, indeed, transport between two points but what of a change in concept that does away



The giant Columns of Jupiter (below left) built by the Romans in Baalbek, Lebanon; a view of the south porch of St Urbain, Troyes (above); and modern Chicago. Three of the many illustrations in *Developments in Structural form*. By Rowland J. Mainstone. Pp. 350. (Allen Lane: London, February (1975.) £12.00.

with the need for transport between those two points? There is, to my mind, a large and very significant element lacking in systems theory. It is the lack of this element which makes people suspicious of handing over their future to a group of cyberneticians. Stafford Beer himself asks why people should trust the cyberneticians: "Who are we to say we know best and everyone else is wrong? ... It is that we are responsible. We are not responsible because we have been elected to govern affairs; we are responsible because cybernetics, that science of effective organisation, is our profession."

I do not think that will do. That is a blatant cry for a priestly cast who alone know the rites of worship and control. We must admit that societies run by priests have been most effective (Aztecs, Incas, and so on) but we also know that they can get their gods wrong with bloody consequences for everyone. There is no doubt that the author is not seeking power of a cen-

tralised sort and he makes a point of saying that he wants the same sort of regulation that keeps the body temperature at 98.4° F by a method of complex interrelating feedback systems rather than through centralised dictat.

I think that Stafford Beer makes a very good case for systems control and for the doom that awaits society if it takes no heed. But there is a great deal still to be done by the systems people who must learn to design systems that will be accepted and used by society on a basis other than exhortation and threat. Selling a system is also part of the system.

"... The Second Law of Thermodynamics has two forms. One is concerned with the pressure to even out energy: that is the form that belongs to our stereotype conception of the universe. It betokens death. The other form is about information content which leads to greater organisation and increasing complexity. That form betokens life." □

DNA Synthesis. Arthur Kornberg. Pp. 399. (Freeman: San Francisco, 1974.) \$18.00.

This is an updated and expanded version of Arthur Kornberg's earlier book, *Enzymatic Synthesis of DNA*. That was published in 1962 and since then, the field of DNA biochemistry has progressed enormously, but has become, in many ways, more and more complex. So this new book is an attempt by a protagonist of DNA replication to provide an overall review of the biochemistry (as against physiology) of DNA synthesis.

The book has been split into natural chapters, each concentrating on a specific area of interest. The first, on the structure and function of DNA, is a good introduction to the basic physical properties of DNA, and the second is an excellent simplified presentation of nucleotide biosynthesis. It may, however, be said that the section on thymine and thymidine utilisation is perhaps a little brief, especially when one considers the extensive use to which these precursors have been put. A whole chapter is spent discussing *E. coli* DNA polymerase I. (The author's laboratory has, in an enzymological *tour de force* spanning some 15 years, elucidated the detailed enzymology of this enzyme. That work has formed the basis for the characterisation of all other DNA polymerases.) Succeeding chapters describe in turn the two other *E. coli* DNA polymerases, enzymes from other bacteria and bacteriophage-induced polymerases and then the relatively murky field of eukaryote and virus induced DNA polymerases. Later chapters also describe the basic biochemical properties of nucleases and *E. coli* RNA polymerase, although the former is dealt with in a rather cursory manner. That is rather surprising considering the reiterated and necessary emphasis on these enzymes throughout the book. The last chapter addresses itself to nucleotide sequence determination and gene synthesis and manipulation, and considers the social and moral aspects of 'genetic engineering'.

All of that, together with the section on polynucleotide ligase represent an excellent summary of the current (early 1974) knowledge of the biochemistry of nucleic acid enzymes. It is in the realm of DNA replication, however, where the book is more subject to criticism. These chapters present not only the known facts but also the author's personal interpretation of DNA replication and it is not always clear what is generally accepted by workers in the field and what is still under dispute. For example, it is by no means proven that RNA always initiates DNA synthesis. Continued re-

search and the test of time will confirm or disprove Kornberg's thoughts. If they are disproved the book may, because of factual rather than speculative errors, age rather faster than it otherwise ought.

To present an overview of DNA synthesis in such detail in only 400 pages is in itself a remarkable achievement and the result is commendable. Despite a few shortcomings, some of which I have alluded to, the book is particularly welcome to workers in DNA replication in providing a concise and up-to-date compendium of nucleic acid enzymology. In addition, the book has an extremely useful comprehensive bibliography that not only follows the text but is also cross-referenced to both authors and subjects. The style of presentation and the good organisation makes for comprehensible (if occasionally encyclopaedic) reading and as such should also be of value to those with only a passive interest in DNA synthesis.

Ian J. Molineux

Nucleic acids

Physical Chemistry of Nucleic Acids. By Victor A. Bloomfield, Donald Crothers and J. R. Tinoco. Pp. x+517. (Harper and Row; New York and London, 1974.) £12.50.

SPECIALISED monographs are aligned between two extremes: that of the scholarly work with a personal flavour, which serves to lever the jaded research worker from his rut so that he may view the surrounding countryside from a new vantage, and that of the encyclopaedic work that provides little but map references to where he may want to go. This book, tends to the latter extreme and is founded on a necessary compromise.

To cover in a manner comprehensible to the novice the theory of all the experimental methods mentioned, to discuss all the ramifications of their interpretation, and to present even the salient results available in the vast literature on the physical chemistry of nucleic acids and their components, would require many, many volumes. On the other hand, to present only the experimental findings would be incomprehensible to all but the specialist—who is a specialist in all aspects of this subject? As a compromise the reader is referred to standard texts for standard theories, and only those not easily found are discussed in detail.

The opening chapters deal with the properties of the constituent bases, nucleosides and nucleotides and with attempts to account for their spectro-

scopic and thermodynamic properties in quantum mechanical terms. The book progresses to a consideration of single stranded oligomers and polymers before coming to grips with various aspects of double helical complexes. A final chapter deals with transfer RNA. Despite this orderly progression, the preoccupations of the authors are sometimes in evidence and readers fascinated by the intricacies of the interpretation of the properties of RNA may be disappointed by the emphasis given to double stranded DNA. The absorption, rotation and scattering of radiation by nucleic acids are well covered though because of the compromise on which the book is founded some fierce quantum mechanical formulae occur with but terse discussion of their meaning, in a way that may well deter the novice. Similar strictures apply to parts of the discussions on hydrodynamic properties and other topics. So encyclopaedic is the range of topics covered that the few sins of omissions may be excused: there is scant mention of electrophoretic properties even though electrophoresis in polyacrylamide gels is now a favourite tool of the nucleic acid investigator; similarly there is no mention of photochemical matters.

The reader will require a background knowledge of physical chemistry, though the level of mathematical expertise required should be well within the range of all likely to want to read this book. Specialists, however, may cavil at the treatment of some topics: thus the section on polymer statistics makes no mention of the rotational isomerism theory; perhaps that is because it is at this point that the theory becomes intricate and not suitable for inclusion in a general text of this sort. Similarly, the treatment of helix coil transitions, though one of the few available at this level, is elementary, and the notion of 'partition function' barely mentioned, and at that in awe inspiring italics. These simplifications are in jarring contrast to some of the quantum mechanical formulations which occur elsewhere.

Despite these criticisms this is a very useful book indeed and is the only volume of this scope that has been published in English. The text is clear, and only occasionally does the numbering of equations and figures go astray. Although the references do not go beyond about 1970, I read the book with much profit and would expect both research students and experienced workers to do likewise. I may not remember all that I have read but at least I now know where to find it all. It is worth the price, high as it is, for that reason alone.

E. G. Richards

Keeping healthy . . .

Viral Immunodiagnosis. Edited by Eduard Kurstak and Richard Morisset. Pp. xiv+334. (Academic: New York and London, October 1974.) \$39.50; £18.95.

THE current lack of specific treatment for most viruses has discouraged the demand for rapid and accurate diagnosis of viral infections. Although precise diagnosis is essential from the epidemiological point of view, the results are generally obtained too late to be of practical value for the immediate care of the patient. Nevertheless, the rapid identification of viruses is yielding important information on the role of viruses in infections of the foetus and the newborn; on the prevention of transmission of hepatitis B by blood transfusion and the spread of this infection by other routes; for the diagnosis of acute and chronic infections of the nervous system; and on respiratory infections, rabies, the herpes group of viruses and others. Similarly, the availability of rapid, specific diagnostic methods is playing a major role in the experimental investigation of a variety of viruses, including the tumour viruses.

The labelling of antibodies with enzymes offers a new technique for application in diagnostic virology. The immunoperoxidase method is a specific, rapid and relatively simple technical procedure which allows the localisation by light microscopy and by electron microscopy of viral antigens present in specimens collected directly from a lesion or from infected tissue cultures. Immunofluorescence and immunoferritin are other methods which provide rapid and specific diagnosis. The identification of viral antigens and antibodies by immune electron microscopy has been used successfully not only for

the investigation of immune complex formation and the serotyping of viruses but also for the detection of viral agents which cannot be easily cultured and for determining antigenic relationships of newly discovered viruses.

The practical procedures for the preparation of enzyme labelled antibodies, for the conjugation of fluorescein, ferritin and cytochrome *c* and the application of these techniques are described in detail in this book in a series of authoritative articles by a number of distinguished contributors. Immunodiagnostic techniques offer new methods for the rapid identification of many viruses and these techniques can be readily applied in hospital laboratories as well as in research laboratories. This book thus fulfills an important need by providing a lucid and a reasonably comprehensive guide, drawing attention to current findings and pointing to new directions of research for the clinical diagnosis of viral infections. An example of future prospects is the technique of cytohybridisation which, although not immunological in nature, offers the advantages of relative rapidity and ease and the reliability with which viral genome can be detected whether or not infectious virus is present. The preparation of specific probes requires a relatively sophisticated laboratory but no doubt such reagents will become available commercially in due course.

The book is well written, with a text that is lavishly illustrated and practical instructions that are explicit and complete, and I therefore wholeheartedly recommend it as essential reading for microbiologists and immunologists. It is a pity that in these days of economic stringency the high price may well keep this useful and stimulating volume away from the bench of many laboratories, where it should clearly be readily available.

Arie J. Zuckerman

Ultrasonic Imaging and Holography Medical, Sonar and Optical Applications. Edited by G. W. Stroke, W. E. Kock, Y. Kikuchi, J. Tsujiuchi. Pp. xi+642. (Plenum: New York and London, 1974.) n.p.

THIS is a fascinating book for all who are interested in remote sensing with non-optical waves. A distinguished group of authors, including Denis Gabor, have contributed to this volume. It comprises a complete set of papers on the subject of holographic imaging and information processing, which were presented at the United States-Japan Science Cooperation Seminar in Hawaii, in January 1973. Most of the 20 papers are concerned with the imaging of human tissue, using ultrasound.

The remaining papers report recent developments in acoustic microscopy and optical signal processing of acoustic images. It is clear from this book that ultrasonic imaging is emerging as a particularly useful technique for application in the medical field, as it is a powerful aid to non-invasive early diagnosis of cancer. The book is therefore to be especially recommended to medical physicists, radiographers and clinicians who wish to be aware of the recent developments in this field.

The diagrams and photographs, so essential to the presentation of this subject, are generally quite good although they have not been standardised by the editors. But that is to be expected in what are really conference proceedings.

A. P. Anderson

Parasites in the Immunized Host: Ciba Foundation Symposium. Pp. viii+280. (Elsevier/Excerpta Medical: London, Amsterdam, New York, 1974.) \$16.20.

UNLIKE the majority of bacterial and viral infections, parasites are rarely eliminated totally from their hosts by the action of immunity. Chronic parasitic infections are the outcome of a delicate balance between the immunological forces of the host on the one hand and the circumvention of these forces by the parasite on the other. The purpose of the Ciba Symposium held at the Ciba Foundation in London from November 13 to 15, 1973 was to discuss the various mechanisms by which parasites are able to evade the immune responses of the host; twenty six eminent specialists in the fields of immunology, parasitology and tropical medicine took part.

There are probably several distinctive ways in which parasites survive in the immunised host. Five mechanisms were examined in detail by experts in those particular areas of research. Antigenic variation, which is thought to be a major factor in the survival of parasitic protozoa, was discussed, particularly in plasmodia and trypanosomes. From the analogy provided by the extensive studies on *Paramecium* it seems likely that a switch in gene activity is the mechanism. A form of variation has been described in *Nippostrongylus* but the mechanism in helminths may be different from that in protozoa.

Certain parasites seem to survive by modifying the immunological responsiveness of their hosts; there are several ways in which this could happen. For example, T-cell responsiveness is dampened in lepromatous leprosy and diffuse cutaneous leishmaniasis and antigenic competition is a possible reason for the poor immunological responses in patients with malaria. Antigens of the host-type, which are related to the blood group substances and appear on the surface of schistosomes are thought to protect this particular parasite from the action of antibodies. Many parasites release soluble antigens and it is possible that these substances facilitate the survival of the parasite by such means as inducing tolerance, stimulating suppressor T cells or blocking cytotoxic mechanisms. The ability of certain parasites to survive within macrophages is, perhaps, the most remarkable example of immune evasion. In some cases this may be because the lysosomes are prevented from fusing with the phagosomes containing the organism, but that is not always the explanation.

Each paper presents an excellent review of up to date knowledge of one aspect of this subject and is followed by a full transcript of the discussion it

provoked. Because of the contributions made by immunologists—unfamiliar with, but nevertheless fascinated by parasitology—the discussions are particularly valuable to those more familiar with the parasite systems. It is only recently that we have come to appreciate the ability of parasites to evade immunity and it is not surprising that more questions were posed than answered at this symposium. It is suggested in the introduction, however, that within the next 20 years we shall have successful immunoprophylaxis of many parasite diseases. Immunologists searching for more practical areas of research would be well advised to read this book.

S. R. Smithers

Proceedings of the 6th Berkeley Symposium on Mathematical Statistics and Probability. Vol. 4: Biology and Health. Edited by L. M. Le Cam, Lerzy Neyman, and Elizabeth L. Scott. Pp. xvi+353. (University of California Press: Berkeley, Los Angeles and London, 1972.) £11.50.

THE Proceedings of the Berkeley Symposia are too well-known to require much introduction. Volume IV (Biology and Health) of the Sixth Symposium contains 25 papers divided into five sections covering "Clinical Trials and Sequential Procedures", "Population studies and branching processes", "Biostatistics", "Cellular Phenomena and Carcinogenesis" and "Psychological Aspects of Observational Studies".

Although the papers on clinical trials and sequential methods were interesting, the important distinction between the two objectives of clinical trials (decision making and investigation of the natural history of diseases) was not made. The extent to which the decision orientated, sequential design can satisfy the second objective is arguable and it would have been valuable to have some discussion of that point. Another basic problem of the application of sequential designs in clinical trials is their dependence on a single response variable both for the stopping rule and the final decision. In many trials situations, however, particularly with chronic diseases, there is no single response variable of major interest. Instead, a battery of responses, of more or less equal status, are noted. To use a sequential design in this situation implies that one response variable must be chosen subjectively as being that on which the sequential procedure depends. Some discussion of these and other difficulties would have given a better balance to this section.

Although most of the papers give interesting mathematical treatment to realistic biological problems, the relevance of some of the papers to *Biology and Health* is not obvious, a pity in a volume with this title. J. A. Anderson

... growing old

Intrinsic Mutagenesis. A Genetic Approach to Ageing. By Sir Macfarlane Burnet. Pp. ix + 244. (MTP Medical and Technical Publishing: Lancaster, 1974.) £6.75.

OVER the years since gerontology became a recognisable discipline, there seem to have been almost as many theories of ageing as there have been workers in the field. This top heavy development, and the relative dearth of contributions from the more renowned workers in those research fields which have a bearing on the ageing process, may well have been responsible for the very tardy award of any accolade of respectability to gerontology. Recently, however, writers such as Sir MacFarlane Burnet have entered the field and are doing much to elevate age research to its proper place among the biological sciences. This book cannot but continue this highly desirable process.

There are two broad theories of ageing, both of which have been under continuous discussion over the past decade. On the one hand are the theories that ascribe programmed origins to the ageing process; on the other hand, the theories that ascribe stochastic or random origins to that process. Sir MacFarlane considers both theories, and develops a new concept in which parts of both have a role to play. His present theory is based on the belief that a certain degree of mutation is required to provide the optimal introduction of the new information necessary for a species to survive in a continuously changing environment. At

the same time such mutation defines the typical lifespan of the somatic cells and the organism as a whole.

In many respects this monograph provides the biological counterpart to Burch's more mathematical treatment of the same problem (*An Inquiry Concerning Growth, Disease and Ageing*; Oliver and Boyd, Edinburgh, 1968), but Sir MacFarlane has been able to draw on the mass of material which has been published since 1968. Of the references quoted, 60% are drawn from works published during the last six years. And Sir MacFarlane's approach is an improvement on that of Burch, in that it discusses the involvement of environmental factors.

Drawing facts from studies of both microbiological and mammalian cell lines, Sir MacFarlane suggests that errors introduced into DNA by faulty expression of those enzyme systems which control the repair of the nucleic acid chains may determine not only the life span of the organism, but also the incidence of age-mediated lesions. These, because of failures in the normal surveillance mechanisms of the body—age related reductions in T and B cell populations—may induce a degree of error accumulation similar in many respects to that which, according to Orgel, results from faulty protein synthesis.

Once again Sir MacFarlane has presented us with a monograph which is both thought provoking and on which future experimental work may be based. All-in-all this book is well worth reading, and it will be quoted often as the study of the ageing process develops.

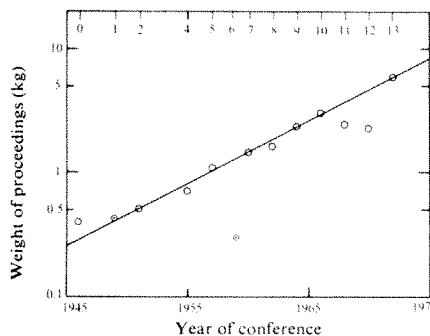
David A. Hall



Aonyx capensis, the cape clawless otter. From *The Carnivores of West Africa*. By D. R. Rosevear. Pp. vii+548+11 plates. (British Museum—Natural History: London, 1974.) £18.50.

*Weighing words in conference proceedings

"I predict for the suitably apocalyptic year of 1984 that... LT 17 will take place, its proceedings will weigh half a hundredweight, and they will take over five years to come out."



THE series of international conferences on low temperature physics has come to be known as 'LT' conferences. The accompanying graph is a log-linear plot of the weight of those LT Proceedings which I have access to, as a function of time and showing the LT number (given along the top abscissa). (On my reckoning, incidentally, the first LT was held at Cambridge in 1946 and not, as the Organising Committee of LT 13* state, in 1949; it is a very fascinating work, well worth reading, and should perhaps be incorporated into the numerology as LT 0). There are several anomalies which can be readily explained. LT 11 was even more anomalous than is obvious from the graph, for a combination of reasons: it came out quickly (within about five months), quite cheaply (about \$17.00 for two volumes) and included some useful discussion. Other people may choose to draw different lines through the points on the graph, depending on their judgement of possible weighting factors. From my own exponential growth line (and some other input data), I predict for the suitably apocalyptic year of 1984 that, unless some serious steps are taken, LT 17 will take place, its proceedings will weigh half a hundredweight, and they will take over five years to come out.

If it had ever been worthwhile—or possible—to review in adequate detail the contents of these four volumes, which it probably never was, it is certainly not worth while now. The LT 13 conference took place in Boulder, Colorado, well over two years ago (August 1972), and the proceedings have only been saved from the ultimate embarrassment of not appearing before the next conference by the fact that LT 14 will take place three years after LT 13 instead of two, as was the previous custom. Colleagues and friends who have been responsible for producing this almost unbelievably ex-

pensive work of supererogation have had a very hard time of it indeed—a somewhat hilarious time too (for those who were not involved, at least)—which included the hijacking of the plane carrying some of the manuscripts, and its destruction in a Middle Eastern desert. They are to be congratulated and commiserated with on a task which, as far as I am concerned, will be thankless.

Is it really worth all the effort and cost to produce in four beautiful volumes all these contributions which, if valuable enough ought to be made more readily available in regular journals, and if not, ought to sink unobtrusively into oblivion? Short conference contributions are not usually, and certainly should not necessarily be, as complete as papers published in the normal way, and one is never certain when referring to them that they are quite reliable. If they are complete and reliable, and are not published elsewhere because the authors consider them to be published already as proceedings, they are often ignored or forgotten.

The four volumes reviewed here contain more than 500 papers on a vast range of topics—all low temperature aspects of quantum fluids and solids, metals, instrumentation, techniques, and so on—totalling over 2,500 pages. The admirable aim of the organisers was to allot half of the available time to plenary sessions dealing with new developments but of interest to a general audience. The plenary lectures were certainly successful but because of the large number of parallel sessions with contributed papers the plenary papers form only about 3% by number and 6% by pages of the proceedings. In addition, there were special sessions, evening sessions, and discussions, much of which were more interesting and valuable than the contributed work, but not recorded. It is very unfortunate, for example, that probably the most interesting topic, which occupied a considerable amount of private and public discussion time (the newly discovered superfluidity in liquid He³) should be found only cursorily reported

under 'Quantum Crystals'. I should like to suggest that the only rational way of dealing with the record of a conference of this size and diversity is to make available at the conference itself fairly detailed abstracts of the papers and properly to publish as a book only a collection of the invited papers whose authors would be aware that they were contributing to a book and not to ephemeral conference proceedings. Everybody would be saved a lot of time, trouble and probably expense, and the result would be much more useful.

Now that the time and effort have, however, been spent it would be unfair not to say that there are many interesting and useful things in the books, as may readily be found by reading through the list of contents. One really astonishing thing is the enormous amount of work on liquid helium, occupying about a quarter of the whole and quite enough for a conference on its own. None of the material can be used, of course, without recent confirmation by ordinary publication or by private communication, with certain exceptions. The exceptions are the established material in the plenary session lectures (each only slightly longer than a letter in *Phys. Rev. Lett.*, although the talks themselves lasted nearly an hour); and, most interestingly from an historical point of view, A. A. Abrikosov's address as recipient of the Fritz London Award (given, alas, *in absentia*) which contains a brief though generous account, which I had come across only by hearsay before, of the way in which Landau, perhaps unintentionally, discouraged him in 1953 from publishing his discovery of vortices in the mixed state of Type II superconductors.

So the conclusion of all this is that all institutions involved with low temperature physics will, if they can afford it, have to buy these books in order to keep their reference literature complete. But it should never happen again, and I sincerely hope that the Organising Committee of LT 14 take note. See the graph. **D. F. Brewer**

The Social Behaviour of the Bees, by Charles Michener, which was reviewed in *Nature*, **253**, 75; 1975, is available in Britain through Harvard University Press, 126 Buckingham Palace Road, London, SW1, at £12.50.

In the same edition (**253**, 76; 1975) we published a review of *Our Future Inheritance: Choice or Chance?* This book was written by Alun Jones and Walter F. Bodmer, whose names were omitted from the bibliographical details.

**Low Temperature Physics-LT 13*. Vols 1-4. Edited by K. D. Timmerhaus, W. J. O'Sullivan and E. F. Hammel. Pp. xv+669. (Plenum: London and New York, 1974.) \$42.00 each.

obituary

William D. Coolidge, the developer of the modern X-ray tube and the ductile tungsten filament used in electric light bulbs, has died in Schenectady, New York. He was 101 years old.

Born in Hudson, Massachusetsts, Dr Coolidge graduated from the Institute of Technology there in 1896, specialising in physical chemistry and electrical engineering. After obtaining his doctorate in 1899 at the University of Leipzig, he joined the research staff of General Electric. In 1908, he invented a method of working tungsten, one of the most brittle of metals, while it was hot, thus making it possible to draw out the metal into wires much thinner than human hair. The durable thin filaments made by this method led to a more durable light bulb for use in cars and trains, and to more powerful and more portable X-ray equipment. The X-ray tube invented in 1913, the 'Coolidge tube', is still the model on

which modern tubes are based. In this case, a hot tungsten filament replaced a cold aluminium cathode. Among many awards were an honorary MD from the University of Zurich, the Rumford Medal of the American Academy of Arts and Sciences, and the Howard N. Potts Medal of the Franklin Institute. Dr Coolidge will be honoured by the National Inventors Hall of Fame at the Patent and Trademark Office, from which he has received 83 patents. In 1932, he was appointed director of the General Electric Research Laboratory. Under his direction, many new discoveries were made, including the development of the sodium vapour lamp, high-quality magnetic steel, improved ventilating fans and the electric blanket. On the occasion of his 100th birthday, he was presented with a huge birthday cake lit with 100 ductile-tungsten lamps and topped with part of a large X-ray tube.

William Charles Osman-Hill, the primate anatomist, has died at the age of 74.

After graduating from Birmingham University in 1925 with an MD, Dr Osman-Hill became an assistant lecturer in zoology there and later lecturer in Anatomy from 1925-30. He was professor of anatomy in Ceylon University until 1944 and became a reader in physical anthropology in the University of Edinburgh from 1945-50. From 1950-62, he was Prosector to the Zoological Society of London and from 1962 until the time of his death he was one of the Trustees of the Hunterian Collection in the Royal College of Surgeons of England, of which he was also a Fellow. Dr Osman-Hill will be remembered particularly for his work on primate anatomy, about which he has published several books, and for many enlightening papers on primate evolution and anthropology.

announcements

Awards

Michel Crozon and **Peter Sonderegger** have been awarded the **Prix Scientifique** by the Fondation de France, for their work on 'Physics for the Man in the Street' at Aix-en-Provence in 1973.

John Miller Meek has been awarded the **Faraday Medal**, for research into electrical discharges in gases, and in particular the mechanism of the electric spark.

E. Kodicek has been awarded the **CIBA Medal and Prize** by the Biochemical Society for contributions to the study of metabolism and mode of action of cholecalciferol in animal tissues.

D. R. Trentham has been awarded the **Colworth Medal** by the Biochemical Society for his work on the kinetics of enzyme action and in particular his contribution to the transient kinetics of myosin-ATPase.

Appointment

Kenneth Dolder has been appointed to the chair of atomic physics at the University of Newcastle upon Tyne.

International meetings

March 13-14, **Chromosomal Proteins and their Role in Regulation of Gene Expression**, Florida (Florida Colloquium on Molecular Biology, Department of Biochemistry, University of Florida, Gainesville, Florida 32610).

April 7-9, **Food from Waste**, Weybridge, Surrey (The Secretary, National College of Food Technology, Weybridge, Surrey, UK).

April 13-16, **Energy and Environment** California (Betty Peterson, Executive Director, Institute of Environmental Sciences, 940 East Northwest Highway, Mount Prospect, Illinois 60056).

April 14-17, **Magnetics**, London (Intermag 75, Institute of Physics, 47 Belgrave Square, London SW1X 8QX, UK).

April 21-25, **Nuclear Energy**, Paris (P. Zaleski, CNE-Inscriptions, B.P. No. 27 92140 Clamart, France).

May 6-8, **Quantitative Magnetospheric Models**, Southern California (W. P. Olson (Chairman), McDonnell Douglas Astronautics Company, 5301 Bolsa Avenue, Huntington Beach, California 92647).

May 11-14, **Ozone for Water and Wastewater Treatment**, Montreal, Canada (The Symposium Chairman, 24 Central Avenue, Waterbury, Connecticut 06702).

May 11-16 **Calcium Transport in Contraction and Secretion**, Padova, Italy (F. Clementi, Istituto di Farmacologia, Università di Milano, 32 via Vanvitelli, 20129 Milan, Italy).

May 12-14, **Transplantation and Clinical Immunology**, Lyon (Secretariat, Docteur R. Triaux, Fondation Mérieux, 17 rue Bourgelat, 69002 Lyon, France).

May 12-16, **Acid Precipitation and the Forest Ecosystem**, Columbus, Ohio (Dr Leon S. Dochinger, US Forest Service Laboratories, P.O. Box 365, Delaware, Ohio 43015).

Reports and publications

Great Britain

- The Soal-Goldney Experiments with Basil Shackleton: a Discussion. By Christopher Scott, *et al.* (Proceedings of the Society for Psychical Research, Vol. 56, Part 209, October 1974.) Pp. 41-174. (London: Society for Psychical Research, 1974.) [1612]
- University of Hull. Annual Report, 1973/1974. Pp. v + 115. (Hull: The University, 1974.) [1612]
- UKAEA, Harwell. Radioactive Fallout in Air and Rain: Results to the Middle of 1974. By R. S. Cambray, J. D. Eaking, Miss E. M. R. Fisher and D. H. Peirson. Pp. 48. (London: HMSO, 1974.) £1 net. [1612]
- Report of the Social Science Research Council, April 1973-March 1974. Pp. 66. (London: HMSO, 1974.) 71p net. [1812]
- Social Science Research Council. Research Supported 1974. Pp. 196. (London: Social Science Research Council, 1974.) 90p net. [1812]
- The British Library, First Annual Report 1973/1974. Pp. 16. (London: The British Library, Store Street, WC1, 1974.) [1912]
- Natural Environment Research Council. Report of the Council for the period 1 April 1973-31 March 1974. Pp. v + 153 + 15 photographs. (London: HMSO, 1974.) £1.50 net. [2012]
- The Mental Health Trust and Research Fund. Annual Report for 1974. Pp. 28. (London: The Mental Health Trust and Research Fund, 8 Wimpole Street, W1, 1974.) [2012]
- Department of Agriculture and Fisheries for Scotland. Farming the Red Deer. (The first report of an investigation by the Rowett Research Institute and the Hill Farming Research Organization.) Pp. 93 + 14 plates. (Edinburgh and London: HMSO, 1974.) £1.70. [2012]
- Proceedings of the Royal Irish Academy, Vol. 74, Section A, No. 15: Paired Homotopy Type. By T. Porter. Pp. 103-114. 32p. No. 16: On Separation Axioms Weaker Than T_1 . By D. M. G. McSherry. Pp. 115-118. 18p. No. 17: The Virtual Theorem and Schwinger's Variational Principle in Collision Theory. By J. D. G. McWhirter and B. L. Moiseiwitsch. Pp. 119-131. 36p. Vol. 74, Section B, No. 25: Granophyre Net-Veining in the Dolerites of Slieve Gullion. By R. W. D. Elwell, P. M. Bruck and P. J. O'Connor. Pp. 439-453 + plate 14. 32p. No. 26: Anticancer Agents—X. Cyclisation of 1-Acyl-, 4-Alkylthiosemicarbazide Derivatives to 1,2,4-Triazoline-3-Thiones in the Presence of Hydrazine. By G. N. O. Callaghan. Pp. 455-461. 16p. No. 27: *Amphitholina cucullus* (Stebbing), a Littleknown Marine Amphipod Crustacean New to Ireland. By A. A. Myers. Pp. 463-469. 15p. (Dublin: Royal Irish Academy, 1974.) [2312]
- Wildfowl 25. Edited by G. V. T. Matthews and M. A. Ogilvie. Pp. 176. (Oxford and London: Blackwell Scientific Publications, 1974. Published for the Wildfowl Trust, Slimbridge.) [2312]
- Bulletin of the British Museum (Natural History). Entomology. Vol. 31, No. 5: The *Nesothrips* Complex of Spore-Feeding Thysanoptera (Phlaeothripidae: Idolothripinae). By L. A. Mound. Pp. 107-188. (London: British Museum (Natural History), 1974.) £4.80. [2412]
- Office of Population Censuses and Surveys. The Current Tempo of Fertility in England and Wales. By Dr. S. M. Farid. (Studies on Medical and Population Subjects, No. 27.) Pp. 95. (London: HMSO, 1974.) £5.30 net. [3112]
- Ministry of Agriculture, Fisheries and Food. Agricultural and Food Statistics: a Guide to Official Sources. (Studies in Official Statistics, No. 23.) Pp. vi + 84. (London: HMSO, 1974.) £2 net. [3112]
- Methods of Chemical Analysis of Iron and Steel. Pp. xi + 161. (Sheffield: British Steel Corporation, Corporate Development Laboratory, 1974.) £4. [3112]

Other countries

- The Walter and Eliza Hall Institute of Medical Research, 1973/1974. Pp. 152. (Parkville, Victoria: The Walter and Eliza Hall Institute of Medical Research, 1974.) [2511]
- CERN—European Organization for Nuclear Research. CERN 74-22: Proceedings of the 1974 CERN School of Physics, Winternice, England, 16-29 June 1974. Pp. vii + 215. (Geneva: CERN, 1974.) [2611]
- Publications of the World Health Organization, 1968-1972—a Bibliography. Pp. 158. (Geneva: WHO; London: HMSO, 1974.) Sw.fr.20. [2611]
- World Health Organization. Public Health Papers, No. 58: Suicide and Attempted Suicide. Edited by Eileen M. Brooke. Pp. 127. (Geneva: WHO; London: HMSO, 1974.) Sw.fr.8. [2711]
- North of Latitude Eighty: The Defence Research Board in Ellesmere Island. By G. Hattersley-Smith. Pp. ix + 121. (Ottawa: Information Canada, 1974.) \$6.75. [2112]
- Geological Survey of Canada. Guide to the Geology of Riding Mountain National Park and Its Vicinity: History of Its Upland and Other Scenery. By A. H. Lang. (Miscellaneous Report No. 20.) Pp. 68. (Ottawa: Information Canada, 1974.) \$2. [2112]
- Bulletin of the American Museum of Natural History, Vol. 154, Article 1: The Eyeless Beetles of the Genus *Arianops* Brendel (Coleoptera: Pselaphidae). By Thomas C. Barr, Jr. Pp. 1-52. (New York: American Museum of Natural History, 1974.) \$2.25. [2112]
- Lawrence Berkeley Laboratory: Research Highlights 1974. Pp. 60. (Berkeley, California: Public Information Department, University of California, 1974.) [2112]
- Smithsonian Contributions to Paleobiology, No. 20: Ultrastructural Studies on Graptolites, I: The Periderm and Its Derivatives in the Dendroidea and in *Mastigograptus*. By Adam Urbanek and Kenneth M. Towe. Pp. 48. (Washington, DC: Smithsonian Institution Press, 1974. For sale by US Government Printing Office.) \$1.35. [2112]

Person to Person

Animals in Research. Professor D. H. Smyth is to carry out an investigation into the contribution made to medical problems by tissue culture, computers and other methods not involving living animals, at the suggestion of the Research Defense Society. He would welcome suggestions and information from individuals or societies (MRC Unit for Metabolic Studies in Psychiatry, Middlewood Hospital, Sheffield, UK).

Youth and Universe. The West Yorkshire branch of the British Association of Young Scientists is organising a four-day programme of lectures and films on the theme 'The International Animal'. This gathering of several hundred school and university/college students from April 1-4 at the University of York will consider such topics as the evolution of man and the future of man in the universe. Contact (before March 1): Bootham School, York YO3 7BY, UK).

Romantic Science. Researcher interested in renaissance of artistic activity during post-Napoleonic or Romantic Period in Germany and Austria (mid-19th to early 20th century) would be grateful for any information about similar developments at that time in science (Robbie Vickers, 49 Barry Road, East Dulwich, London SE22, UK).

Egyptian Medicine. Student of Egyptology at Oriental Institute, Free University, Brussels, specialising in problem of 'doctor-medicine preparer' (his role, place in society, knowledge), and of Egyptian pharaonic medicine in general, wishes to communicate with doctor interested in research in history of medicine (Martine La Graviere, 10 rue Philippe Dewolfs, B1170 Bruxelles, Belgium).

Irish Astronomy. Any information on Irish megalithic sites would be gratefully received (Lillian Thompson, Flat 3, 14 College Terrace, Brighton BN2 2EE, Sussex, UK).

Flat or small house. Required for a week at the end of July in the vicinity of Bruges, Ghent or Rheims. Two adults and baby (R. E. Woodham, 167 Crofton Road, Orpington, Kent BR6 8JB, UK).

There will be no charge for this service. Send items (not more than 60 words) to Robert Vickers at the London office. The section will include exchanges of accommodation, personal announcements, and scientific queries. We reserve the right to decline material submitted.

- Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft, Berlin-Dahlem. Heft 160: Untersuchungen über die Anfälligkeit verschiedener Getreidearten gegen den Erreger der Schwarzbeinigkeit, *Ophiobolus graminis* Sacc. Von Dr. Horst Mielke. Pp. 61. (Berlin-Dahlem: Biologische Bundesanstalt für Land- und Forstwirtschaft, 1974.) DM9. [312]
- World Health Organization Technical Report Series, No. 556: Detection of Dependence-Producing Drugs in Body Fluids—Report of a WHO Meeting of Investigators. Pp. 50. (Geneva: WHO; London: HMSO, 1974.) Sw. fr. 5. [412]
- Annals of the South African Museum, Vol. 65, Part 10: The Cranial Morphology of *Thrinaxodon liorhinus* Seeley. By S. Fourie. Pp. 337-400. R.8.40. Vol. 65, Part 11: Aspects of the Biology and Ecology of the Genus *Tylos* Latreille. By Brian Kensley. Pp. 401-471. R.8.70. Vol. 66, Part 1: Population Structure in *Agama atra* and *Cordylus cordylus* in the Vicinity of de Kelders, C.P. By Bryan R. Burrage. Pp. 1-23. R.4. Vol. 66, Part 2: A New South African Representative of the South West African Genus *Namibinyndus* Hesse (Diptera: Mydidae), with some Ecological Notes on the Habits of the Species. By A. J. Hesse. Pp. 25-34. R.2. Vol. 66, Part 3: The Cranial Morphology of the Lower Triassic Dicotylid *Myosaurus gracilis*. By Michael A. Cluver. Pp. 35-54. R.3.80. (Cape Town: South African Museum, 1974.) [512]
- Preliminary Report on the Izu-Hanto-oki Earthquake of 1974. (Special Bulletin of the Earthquake Research Institute, University of Tokyo, No. 14.) Pp. 255. (Tokyo: Earthquake Research Institute, University of Tokyo, 1974.) [612]
- Twenty-second Annual Report of the Australian Atomic Energy Commission, 1973/1974. Pp. 117. (Coogee, NSW: Australian Atomic Energy Commission, 1974.) [912]
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nature

February 27, 1975

Are summit agreements of much use?

WHATEVER the outcome of top-level meetings that heads of state seem to delight in, the agreements on scientific, technical and medical cooperation can always be relied on as a stand-by. If the discussions were cordial, wide ranging and in an atmosphere of mutual understanding then there were probably plenty of good things to emerge: grain deals, trade credits, non-aggression treaties and the like are bound to eclipse anything on the scientific side. But if the discussions were merely correct, a helpful exchange of views and in an atmosphere of mutual respect, then scientific agreement is wheeled out as a major symbol of that mutual respect, and some hare-brained joint projects are launched. Part of the deal often consists of trips for the presidents of the respective academies around endless laboratories of their counterpart's country. Few scientists have not had at some time or other to conduct an immensely bored (with great justification) scientific luminary on a fifteen minute tour as the result of some cosmetic deal concluded ages before by heads of state.

Mercifully, Mr Wilson returned from Moscow last week with a bagful of deals to present to the public, so we were spared Anglo-Soviet cancer projects, joint expeditions to the South Pole and so on. And yet although not eye catching, there are agreements of interest to the scientist, although you'll have a job finding out much about them, as not many people in Whitehall seem to know very much about what went on.

Perhaps the most bizarre idea to emerge was of a round-table conference of academics. Apparently someone (Harold Wilson, one suspects) thought it a good idea to put together a diversity of 'distinguished representatives of public life, science, culture, commerce, the press and other fields' from the two countries. When in Britain they will meet under the auspices of the Royal Institute for International Affairs, which thought it a bit premature to say anything other than that the concept has not progressed very far yet. How this strange group will be 'selected', what it will talk about and what good will be done by it has yet to be spelt out. We shall follow its career with the mildest of curiosity.

An almost equally outrageous idea that found its way into the communique was that the Soviet Union and Great Britain would collaborate on attempts to keep to a minimum the number of underground nuclear tests; this is no doubt meant to be seen as a positive gesture by two of the three guarantors of the Non-Proliferation Treaty.

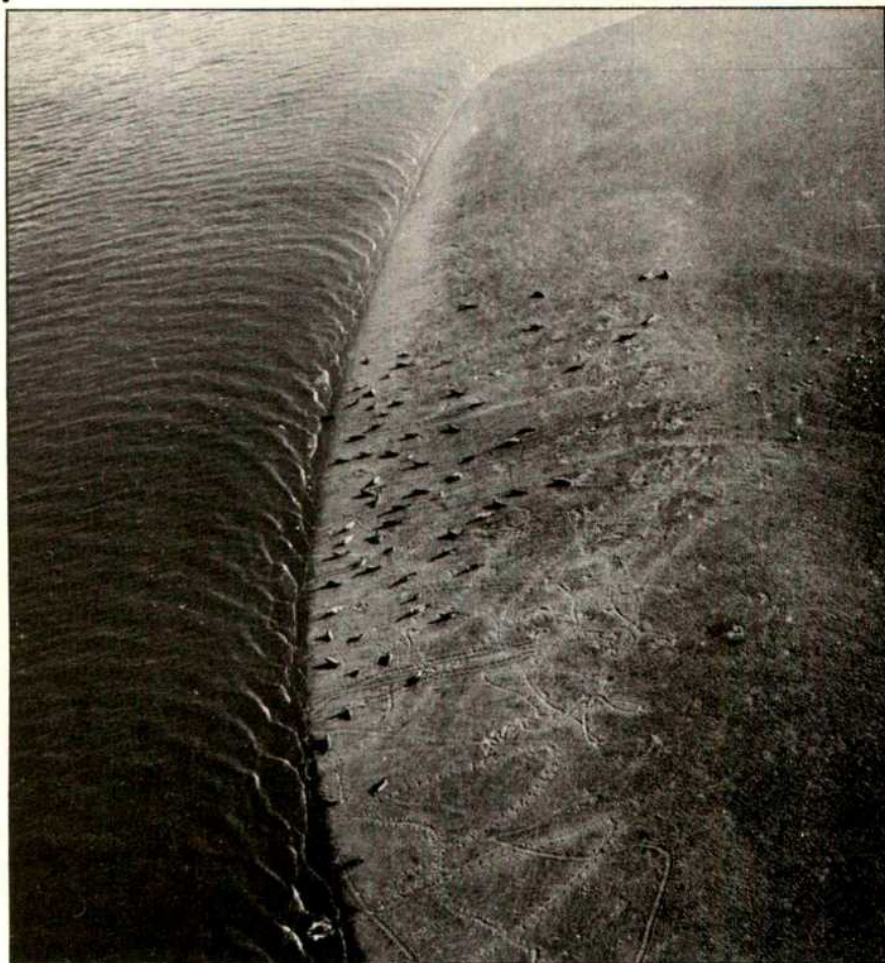
Since the British government has got out of the habit of acknowledging to the public its nuclear activities and since the Soviet government never got into the habit in the first place, they have both, in a sense, already kept things down to a minimum. Unless these two arch rivals start sharing their top-secret data, it is hard to see how the commitment is other than pure humbug.

On scientific and technological cooperation *per se*, the communique does little beyond pat on the back two inter-governmental commissions the existence of which we had all but forgotten. The commission on applied science, technology, trade and economic relations comes up with encouragement for joint efforts in (among other things) high temperature plasma physics, astronomy and applied microbiology, although it is difficult to see much rhyme or reason in the list.

What do scientists really want from meetings of heads of state? Nobody seems to ask the community before visits, and certainly it is hardly the easiest thing to find out to whom one should talk if one feels strongly. It is perhaps foolish to look for anything from bilateral talks; science, as opposed to big technological projects, is nominally an international affair. Nonetheless, there are some matters of principle that could have been raised. If science is indeed international and one works in a spirit of trust that transcends frontiers, accessibility is the key to scientific development. Scientists tend to rely fairly heavily, in developing their own ideas, on the work of those other scientists whom they either know and have visited or at least whom have heard favourably about from a colleague.

On the whole it is possible to form these contacts almost without limit in many countries, and the better the telephones, airlines and travel budgets the better the opportunities for doing science. The links are palpably inadequate in the Anglo-Soviet situation; exchanges of a few scientists a year do little more than scratch the surface, and the ultimate aim must clearly be to make an Anglo-Soviet agreement as redundant as an Anglo-American one would be. Neither the recent agreement, nor any envisaged in the near future, goes very far in this direction.

If you have any ideas and you don't think you will be invited to the round-table, it is a bit of a problem knowing whom you should contact. Try the International Technological Collaboration Unit at the Department of Trade.



Counting the Common Seal

How many Common Seals are there in the Wash? Is the population in danger of extinction? Conflicting estimates of the size of this stock have attracted considerable public attention since the Home Secretary refused to grant hunting licences in 1974 in spite of the fact that 400 could legally be culled under the Conservation of Seals Act 1970. Dr C. F. Summers of the Seals Research Division (SRD) of the Institute for Marine Environmental Research and Dr M. D. Mountford of the Institute of Terrestrial Ecology, both of the Natural Environment Research Council, explain the basis on which population estimates are made.

THE seal biologist does not have access to samples representative of whole populations in the same way that a fish biologist might, because commercial sealing in Britain concentrates almost exclusively on seal pups which provide high quality skins for the fur trade. It is, therefore, difficult to estimate the size of seal stocks directly.

This problem is usually surmounted by measuring some index of the population, such as the number of pups born annually. This is useful for identifying trends in stock size and, if adequate life-table data for the species are available from another source, it is possible to convert pup production to an all-age estimate (assuming that the life table parameters have similar values in both stocks).

If we consider the problem of obtaining an estimate of annual pup production, the Common Seal, *Phoca vitulina*

vitulina, presents peculiar difficulties. Unlike the Grey Seal, *Halichoerus grypus*, it does not congregate into recognisable breeding assemblies, nor do the pups remain on land for more than a few hours after birth. Direct counting of pups does not, therefore, yield realistic estimates of production. Nevertheless, this is all that can be attempted in some localities and the best information obtainable is a minimum estimate of productivity.

In the Wash, however, where pups are relatively easy to catch for tagging and where the commercial hunters have cooperated by returning the tags, it has been possible to obtain a more realistic estimate of pup production than elsewhere. Before describing this it is interesting to consider earlier attempts to measure the size of the Common Seal stock in the Wash.

Counts of Common Seals hauled out

on sandbanks throughout the Wash have been recorded since the 1920s. Before 1965, however, the counts did not cover the entire area. They were made by individual observers from boats and, even with a very fast boat, it is not possible to visit all the sandbanks in the Wash during the period of a single low tide.

Since 1965 aerial photographic surveys have been made by the SRD at all states of tide and throughout the year, visiting all the sandbanks in a short period of time. Fluctuations in the counts made from these photographs suggest that the isolated counts of a few sandbanks made from a boat cannot be used to estimate the size of the whole population. Moreover, since it is not known what proportion of the population is hauled out at any one time the published counts from the aerial photographs do not give a correct estimate of the number of seals in the Wash. It was suggested in the 1972-73 report of the Universities Federation of Animal Welfare that only a small proportion of seals remain in the water at low tide and that as the maximum number of seals reported hauled out at any one time was 1,722 (July 17, 1969) the population size of around 6,000 reported by the SRD must be a gross overestimate. This argument is invalidated by the very large variation in the aerial counts. For example, in February 1968, 472 seals were counted as compared with 1,534 in March 1968. Between these two observations the population had either trebled through immigration, or a very large proportion of the seals which hauled out in March did not do so in February.

Common Seals shot in the Wash between 1911 and 1953 attracted a bounty paid by the Ministry of Agriculture, Fisheries and Food and the Eastern Sea Fisheries Committee. The animals were killed to protect local fisheries and consisted of juveniles and adults. Commercial seal hunting for the fur trade started in the Wash in the early 1960s and large numbers of pups were taken before the passage of the Conservation of Seals Act 1970. (The Act provides protection for both Grey and Common Seals during their respective pupping seasons but also allows seals to be killed for several different purposes. Seals are killed under licence in the Wash in compliance with that section of the Act which permits "the use of a population surplus of seals as a resource.")

Between 1968 and 1973, the SRD used a capture-recapture technique to estimate Common Seal pup production in the Wash. Pups were tagged in June and early July and an estimate of pup numbers was made from the proportion of tags 'recaptured' in the hunters'

catch, usually in the second and third weeks of July. To give a correct estimate the capture-recapture method requires certain conditions to be met. (1) The tagging and the hunting effort should be random. Older pups are more difficult to tag and shoot, but the chance of being tagged and the chance of being shot, if related at all, must be positively correlated. This has the effect of underestimating the population size.

(2) In the interval between tagging and hunting there must be no gains or losses through immigration, emigration or mortality. Immigration and emigration are not important, since pups move only short distances during the first few weeks of life. If, however, this condition is not met with regard to mortality, the capture-recapture method overestimates the population size by a factor of $1/p$, where p is the probability that a pup, alive at the time of tagging, is still alive and present in the population at the time of hunting. No precise figure of the mortality during the sampling period is available but a 21% post-natal mortality for the first year of life has been reported for *P. vitulina richardi*. Therefore, the upper limit of the degree of over-estimation that could be produced by the capture-recapture method is given by assuming that the whole of this first-year mortality occurs in the interval between tagging and hunting. If this happened the method would over-estimate the population here by about 27%.

(3) There must be no recruitment of pups during the 'recapture' sampling. Although this is not the case, while the method overestimates the population at the time of tagging, it gives an unbiased estimate at the time of hunting.

(4) There should be no difference in mortality between tagged and untagged pups. Since it was found that tagging did not affect the growth rate it is unlikely to have increased pup mortality.

Table 1 shows the pup production estimates calculated for the years 1968-73. There is no value for 1969 because tags were not properly returned, or for 1974 because there was no hunting. When the five years are combined, an estimate and approximate 95% confidence interval of $1,450 \pm 239$ pups is obtained. The data suggest neither an upward nor a downward trend in productivity.

Using a life-table for *P. vitulina richardi* in British Columbia compiled by M. A. Biggs (*Bull. Fish. Res. Bd Can.*, No. 172, 1969) it is evident that the number of pups born represents about 1:4.5 of the total stock. The estimate obtained from this table for the all age population, that is pups,



Cow with pup

non-breeding and breeding adults, is $6,525 \pm 1,076$.

We have referred to a possible over-estimation by the capture-recapture technique but, considering other available evidence, it is fairly certain that during the mid-1960s the Wash population was at least 4,000. In 1966, 850 pups were shot by commercial hunters. As it is unlikely that they ever shot an entire year class, the all-age population must have been in excess of about 3,825 (850×4.5) in 1966. This level of hunting would take four or five years, after recruitment of survivors to the breeding stock, to influence pup production. But whatever its effect the population size in 1970 could not have been less than about 3,915 ($4.5 \times$ number of pups shot, see Table 1) and, adding the same proviso about the hunters taking an entire year class, was probably much higher.

Our population estimate is considerably larger than the maximum number of seals counted hauled out together (1,722 on July 17, 1969). Juvenile Common Seals from the Wash have been reported from the Forth estuary to the French coast in tag recoveries. If they occur on any appreciable scale, these movements from the area, perhaps to feed, would mean that a proportion of the total stock was missing from the

Wash haul-outs at any particular time. Local population 'explosions' at places such as nearby Donna Nook, Blakeney and Scroby Sands cause alarm among drift-net fishermen but last for a few days only and suggest that such feeding migration often occur.

The Common Seal in the Wash is clearly not on the verge of extinction as some reports have claimed. In the early 1970s there was a conviction among biologists and seal hunters alike that the heavy exploitation of the Common Seal in Shetland, where around 90% of the annual pup production was taken, would irreversibly reduce the stock if allowed to continue. Consequently a complete ban on Common Seal hunting in Shetland was implemented in 1973. The level of exploitation in the Wash has never reached this high figure. Nevertheless, the provisions of the 1970 Act allowed the introduction of a more restricted hunting regime. The quota was set at 400 pups in 1971, compared with an average of over 700 a year in the six-year period immediately preceding the passage of the Act. The current legislation provides a flexible means of applying controls as they are needed and as long as there is a programme of continuous monitoring, the stock in the Wash should not be at risk. □

Table 1 Capture-recapture data and estimates of pup production

Year	No. of tags applied	No. of tags in catch	Size of catch	Estimate of pup production
1968	37	12	459	1,309
1970	39	22	870	1,419
1971	174	27	303	1,900
1972	111	35	380	1,175
1973	193	36	380	1,987

international news

Less cash for British science

by Eleanor Lawrence

THE Science Budget recently announced for 1975-76 by the Chairman of the Advisory Board for the Research Councils (ABRC), Sir Frederick Stewart, holds little joy for the research councils. There is still a fall in total expenditure of 1% on the figure for 1974-75 and all the research councils except the Social Science and Natural Environment Research Councils will have less money in real terms compared with this year. Even more telling is the observation that the level of spending for 1975-76 represents 6% less than expectations of a few years ago.

The total of £171 million, approved by the Secretary of State for Education and Science but still to be passed by Parliament, is split up into £13.1 million for the Agricultural Research Council (ARC), £28.9 million for the Medical Research Council (MRC), £19.2 million for the Natural Environment Research Council (NERC) and £96.4 million for the Science Research Council (SRC). The Social Science Research Council receives £8.7 million and the remainder goes in grants to the Royal Society and the Natural History Museum.

The ARC, the MRC and the NERC also receive substantial commissions from the appropriate government departments, under the terms of the 1972 White Paper.

But, after the present period of contraction, the ABRC is proposing a period of growth up to 1980, albeit at the modest rate of 3.5% a year for the MRC, the ARC, the NERC and the SSRC, and only 0.7% for the SRC. This reflects the feeling that the time has come to look more closely at the proportion of Science Budget taken up by 'big science' projects (in astronomy and high energy physics for example) which need costly equipment and installations



SMALL MEANS MORE

that will be used by only relatively few scientists. The Mark VA radiotelescope has already been vetoed and the SRC will now have to decide whether it can afford two other projects, the Northern Hemisphere Observatory (estimated to cost £12 million) and the major accelerator project (EPIC) estimated to cost around £25 million.

The ABRC's view is that, in these uncertain times, overall scientific capability should be maintained even at the expense of some of the SRC's prestigious and intellectually exciting

big science. Also, with the decrease in university funding the research councils may find themselves having to pick up bigger bills for the multitude of small projects in universities financed by research council grants but with facilities provided by the university.

Even the cautiously optimistic forward look of the ABRC could fall foul of present and forecast economic crises. The call of certain politicians for decreased public spending generally could erode even the proposed modest rate of growth. □

Annual percentage growth of the Science Budget and research councils' allocations

	1966-67	1967-68	1968-69	1969-70	1970-71	1971-72	1972-73	1973-74	1974-75	1975-76	1976-77- 1979-80
ARC	7.3	12.2	7.3	3.2	7.0	3.1	3.7	4.0	-3.7	-0.6	3.5
MRC	11.6	14.9	5.1	6.9	8.1	3.6	3.6	2.7	-1.7	-1.0	3.5
NERC	17.8	20.6	13.5	20.4	10.4	5.3	4.9	4.4	-3.0	+0.2	3.5
SRC	13.2	6.9	5.4	2.9	3.8	4.8	4.1	3.2	-2.2	-2.2	0.7
SSRC	—	47.3	47.9	25.9	30.3	16.0	10.0	8.6	5.7	+6.8	3.5
Total	13.3	11.2	7.4	6.1	7.1	4.7	4.2	3.9	-2.1	-1.3	2.2

Transfers to departments under the terms of the 1972 White Paper have been included in figures for after that date.

THE full effect of the cuts that President Ford wants to make in federal support for biomedical research has now been calculated by budget officials at the National Institute of Health (NIH). When stated in terms of the number of projects that would go unfunded if Congress allows Ford to have his way, the NIH figures make the situation look horrendous.

The background is that at the end of January President Ford proposed to slice \$351 million from the budget that Congress has already approved for the NIH for the 1975 fiscal year (which is now two-thirds over). Then he followed that suggestion with a budget proposal for the NIH next year which would reinstate only \$72 million of the cut. Congress has to approve both those measures before they can be put into effect, however, and it is unlikely to do so.

One reason is that at the funding level that Congress has already approved for the NIH this year, only about 49% of the projects submitted for NIH support and approved as scientifically worthwhile by peer-review groups would actually be funded. Even that would represent a decline of 4% from last year, but if the proposed cuts are made, only 29% of the approved grants would be funded. And, at the budgetary level proposed for next year, the proportion would sink to a miserable 18%.

That situation arises because a good deal of the NIH's budget is already earmarked to support projects begun in previous years but which still have some time to run. In other words, the axe would fall heaviest on those projects which are now coming up for support, which means that few new ideas would get funded, and biomedical research would be put into something of a holding pattern.

Moreover, if the proposed cuts are made, even continuation grants would be reduced by 5%—a figure which the Administration insists can be absorbed without having to terminate any project, but which will amount to about 13% or 14% when inflation is taken into account.

Stated in term of absolute numbers, the situation is no less depressing. According to NIH figures, at the budgetary level approved by Congress, the NIH could fund about 4,360 competing projects this year (that is, new projects and those coming up for grant renewal) but at the level proposed by President Ford, only 2,320 competing projects would get the green light. For the National Cancer Institute alone, the difference between the Congressionally approved budget and President Ford's proposal amounts to about 620 projects—about 1,200 competing grants would be funded at the Congressionally approved level whereas only 580 would

get the green light under Ford's proposed budget.

With those figures to cogitate upon, Congress, which has a history of being more generous than the Administration toward the NIH, is very unlikely to allow the cuts to be made.

● Meanwhile, in spite of huge uncertainties in the funding of biomedical research, the number of students enrolling in graduate biology departments continues to increase dramatically. According to the latest compilation of figures from the National Science Foundation (NSF), graduate enrolment in the life sciences shot up 17.2% last autumn, and thanks to that surge in numbers, total enrolment in science and

Washington seen

by Colin Norman

engineering courses at graduate schools throughout the United States is higher now than at any time since 1969. Demand for most non-biological courses remained static, however, the only other popular field being the social sciences where enrolment rose by about 4.4%.

The sharp upturn in the number of students taking graduate courses in the biological sciences follows a period of rapid growth in undergraduate biology departments in the past few years. When asked by the NSF if they could explain this fascination with biology, deans and chairmen of biology departments suggested that part of the reason is that many students who fail to get into medical school because of fierce competition are taking up related fields of study instead. Another oft-cited reason is that biology offers a way to "make a contribution to society", the NSF study notes.

Whatever the reason, the upturn in numbers of graduate students in science and engineering departments is welcome news for the universities, which have recently been in dire financial straits. And it is also worth noting that this year's huge increase in enrolments in graduate biology departments has come at a time when many biomedical scientists have been warning that cut-backs in federal support for the biological sciences would dissuade bright young scientists from taking up a career in biological research.

Furthermore, the NSF figures suggest that the number of students taking up engineering courses may have decreased slightly this year, a fact which does not augur too well for the future supply of engineers to carry out the promised expansion of energy research and development in the United States.

● Nearly seven months after atmos-

pheric scientists began to warn that the propellants used in aerosol spray cans could deplete the ozone layer, the federal government has announced that it has established a high level task force to look into the problem. Although slow off the mark, the government is now hoping to move quickly—the task force has been given only four months in which to produce a report.

That will, however, be no easy matter, for the task force has been given a very broad remit. It is expected to "summarise atmospheric, medical and ecological information on the subject, evaluate possible economic impacts and alternatives available to the industry, define potentially applicable authorities under which federal actions can be taken, and outline the proposed federal program to resolve the issue". The task force will, at least, have a wealth of data available from the recently published report on the effects of supersonic aircraft flying in the stratosphere.

At the same time, the National Academy of Sciences has a study in progress on the effect of aerosol spray propellants—fluorochlorohydrocarbons, which are usually referred to by the trade name of Freons—on the ozone layer. The chairman of that study group is Dr Donald M. Hunten, of Kitt Peak Observatory, who has already said at a press conference that in his opinion, the injection of Freons into the atmosphere should be halted as rapidly as possible and the moratorium on their use should last at least until the dangers have been fully evaluated.

Concern over the atmospheric effect of Freons is focused on a theory, published in *Nature* last year by Drs M. J. Molina and F. S. Rowland, of the University of California at Irvine, that the propellants will break down in the upper atmosphere to release chlorine, which will then attack the ozone layer. Because ozone filters out much of the harsh ultraviolet radiation from the Sun, it provides an important protective shield around the Earth; threats to the layer's composition should therefore not be taken lightly.

The chief problem with assessing the effect of Freons on the ozone layer is that because they are chemically very inert, they will drift upwards very slowly, remaining unchanged until they reach the upper atmosphere. Because it may take years for Freons to reach the ozone layer, even if their use is halted as soon as any effect is noticed, depletion would continue for several years after a ban is imposed.

That is one reason for the short time that the task force has been given. Another is that the Consumer Product Safety Commission is being sued by a public interest law firm to ban aerosol spray cans. □

Materials shortage shocks ahead?

by Colin Norman, Washington

The National Academy of Sciences committee report forecasting a bleak future for fossil fuels in the US (February 20) was not limited exclusively to the supply and demand for oil and gas. "Man faces the prospect of a series of shocks of varying severity as shortages occur in one material after another", it suggests, and adds that "the first real shortages (are) perhaps only a few years away."

The overriding conclusion that runs through the 348-page report (prepared by COMRATE—the academy's Committee on Minerals Resources and the Environment) is that more effort is needed to conserve materials and to improve the efficiency with which they are used. "Above all", it states, "we should adopt a conservation ethic that has at its heart avoidance of waste and more efficient use of materials."

● **Copper.** Although deposits of copper within the United States are, in theory, sufficient to meet demand at least until the end of the century, the report notes that its availability will be dictated by success in finding new deposits and that there is also likely to be a shortage of smelter capacity. COMRATE therefore concludes that "we do not foresee continued United States self-sufficiency in production of primary copper and therefore recommend a strong policy of conservation". The only large, unexploited deposits of copper so far identified are manganese nodules on the deep seabed, and the report recommends that "developing the recovery of copper and associated metals from these nodules be encouraged with due regard to the potential impact of undersea mining on the environment". Surprisingly, however, the report has virtually nothing to say on the touchy political question of how exploitation of manganese resources should be regulated—an issue which sharply divided the Law of the Sea Conference last year, and according to most American mining companies, until the political questions are settled at an international level the prospects for deepsea mining are cloudy, at best.

● **Substitutes** need to be found for three materials which have unique uses, and whose supply is threatened, COMRATE suggests. They are helium, which is likely to be in high demand for use as a coolant for superconductors, particularly for transmission lines; mercury, which has no substitute for its use in mercury-wetted switches, and asbestos.

As far as helium is concerned, the



Is it fair to force your baby to smoke cigarettes?



This is what happens if you smoke when you're pregnant.

Every time you inhale you fill your lungs with nicotine and carbon monoxide.

Your blood carries these impurities through the umbilical cord into your baby's bloodstream.

Smoking can restrict your baby's normal growth inside the womb. It can make him underdeveloped and underweight at birth. Which, in turn, can make him vulnerable to illness in the first delicate weeks of his life.

It can even kill him. Last year, in Britain alone, over 1,500 babies might not have died if their mothers had given up smoking when they were pregnant.

If you give up smoking when you're pregnant your baby will be as healthy as if you'd never smoked.

THE 1975 campaign of the Health Education Council to warn pregnant mothers of the dangers of smoking has just begun. Mr Alastair Mackie, the council's Director-General, claims "we do not want to increase the usual anxiety felt during pregnancy . . . [The campaign] exploits the success

achieved last year when research showed that behavioural change followed our advertising. We learned useful lessons, and these will be put into effect in the new project". Above (left): new project; right: the old. Below: new copy. No prizes for spotting a 'useful lesson' learned.

When a pregnant woman smokes she puts her unborn baby's life at risk. Every time she inhales, she poisons her baby's bloodstream with nicotine and carbon monoxide.

Smoking can restrict your baby's growth inside the womb. It can make him underdeveloped and underweight at birth. It can even kill him.

In just one year, in Britain alone, over 1,500 babies might not have died if their mothers had given up smoking when they were pregnant.

If you give up smoking when you're pregnant your baby will be as healthy as if you'd never smoked.

The Health Education Council

report recommends that conservation should be encouraged and that alternatives be found for such applications as arc welding and dispersive uses such as purging rockets and spaceships.

● **COMRATE** further suggests that alternatives be found for chromium, gold, platinum and palladium since those metals are largely concentrated in a few countries, thereby opening up the possibility that the United States could become vulnerable to embargoes or international cartels. It singles out chromium in particular, since virtually all the world's resources are concentrated in Rhodesia and South Africa. In addition, the report suggests that substitutes be found for the use of tin in view of potential world-wide shortages of the metal. The report emphasises, however, that "the discovery and development of new and improved materials as possible substitutes for existing ones takes time and . . . the process is generally driven by clearly perceived functional objectives rather than by ill-placed optimism that 'something will turn up when the crunch comes'".

● **Coal.** Although the report notes that there should be no shortage of coal supplies in the United States for more than a century, it has a few complaints

to make about the adequacy of research and regulations designed to ensure that coal is mined and used more carefully.

As far as mining is concerned, the report suggests that there is an "urgent need" to overhaul methods for detecting, diagnosing and compensating for pneumoconiosis, the so-called Black Lung Disease which claims the lives of thousands of miners, and which in terms of compensation alone may cost up to \$8,000 million a year by 1980.

The report also urges that efforts to mitigate the health hazards associated with the use of coal be stepped up. In particular, it is recommended that more research be conducted on the health effects of small particles, and on the growing problems of acid rain caused by the oxidation of sulphur dioxide to sulphuric acid. As a matter of priority, the report also urges that equipment for removing oxides of sulphur—so called stack gas scrubbers—should be installed in plants where high sulphur fuels are burnt. Several electric utilities, most notably the American Electric Power company, have been fiercely resisting such requirements and have been conducting advertising campaigns running into millions of dollars to try to win public sympathy for their case. □

AFTER a year notable for set-backs, the Soviet space programme has made a successful start to 1975 with the Soyuz-17/Salyut-4 mission. As well as testing out some modifications to the hardware, notably the new autonomic navigation system, the two-man team carried out a wide range of biological and physical experiments.

Although some of these experiments are now of a fairly routine nature—medical tests on the cosmonauts and monitoring the ultraviolet radiation of the Sun, for example—some of them have a more adventurous quality. Among these must be classed the 'Filin' experiment, designed by a team from the Shternberg Astronomical Institute of Moscow State University. This comprises a group of spectrometers for monitoring X-radiation of stellar origin in the 0.2–10 keV range, together with telescopes with a field of view of approximately 1°, intended to correlate the spectrometer readings with visual observations. It is hoped that the results of this project may provide information on white dwarfs and neutron stars. Special attention was given to Rigel and to the remains of the Vela supernova. A somewhat speculative *Pravda* article (January 24) suggests that this experiment may even throw some light on the vexed subject of black holes. During one orbit simultaneous infrared and X-ray observations were made of the characteristics of the background radiation along the Galactic meridian.

Nearer home, the 'Spektr' experiment studied the ambient conditions of the spacecraft, and in particular, the effect of its constant bombardment by charged particles. A special apparatus, produced by one of the Instrumentation Institutes of the Space Research Institute of the Soviet Academy of Sciences, consisted of two subsystems—one to analyse the incident flow of particles and the other to analyse those emitted after impact. These measurements, which were recorded on tape so as not to overload the communications channels to ground control, are intended, not only for use in plotting a quantitative model of the upper ionosphere, but also for the design of future spaceships and orbital craft.

Solar observations, now a routine part of any orbital mission, included a series of spectrography and diffraction spectrometry experiments, which automatically recorded the ultraviolet radiation from the entire solar disk, while at the same time flight engineer Grechko photographed sunspots and prominences.

Later investigations with the solar telescope included an experiment in which protective coatings were

deposited on the surface of the mirrors, in order to assess the possibilities of creating and restoring good optical properties to mirrors subjected to bombardment erosion.

Another routine experiment—the recording of infrared radiation from the Earth and Moon—on this occasion included a special "deep-cooling"

Soviet spacemen's programme

from Vera Rich



Grechko and Gubarev board Soyuz-17.

system "sublimation cold accumulator", to maintain the sensitive element of the infrared telescope-spectrometer at the required temperature. These infrared observations of Earth always form an important part of Soviet orbital missions, since they can be justified in terms of the national economy as a means of surveying resources. In the Salyut-4 programme, however, infrared observations were also used to investigate cosmic phenomena. Salyut 4 carried a special infrared device, the ITS-K, which, in the words of Dr M. N. Markov of the Lebedev Physics Institute of the Academy of Sciences,

can "at the same time seek the 'finger-prints' of the molecular components of matter on the planets and can also look under definite layers of the atmosphere surrounding the planets of the Solar system" (*Pravda*, January 30, 1975). The ITS-K experiment, in fact, is a continuation of a programme begun by the Lebedev Physics Institute, back in 1968, using high altitude (500 km) rocket observations.

Visual photography (both black-and-white and colour) is also traditionally presented by the planners as a valuable contribution to "the study of the environment and the solution of individual problems in the interests of the national economy". On this mission, special emphasis has been placed on such photography, since, in general, the study of such photographs taken in winter has been somewhat ignored. It is hoped that the snow-cover may throw into strong relief ravines and gullies and other features of geological interest. Special attention has been given to the photography of Soviet Central Asia and Kazakhstan, and the south-western European region of the USSR including the Caucasus. Unfortunately for the conservationists, weather conditions prevented photography of the Baikal area.

The on-board experiments (code-named 'Oasis') included a number of genetic, embryological, physiological and biotechnical projects using insects, microorganisms, tissue cultures and higher plants. Special mention is made of *Drosophila* (once legislated out of Soviet science under Lysenkoism) and *Chlorella*. Biological experiments on the cosmonauts themselves included a series of tests monitoring the respiratory and cardiovascular systems using graded loads, a "special vacuum suit" and a rotating chair. On the nineteenth day of the flight, an ultrasonic device was used to determine changes in the density of bone tissues, and blood samples were taken for post-flight analysis.

Also at the biological level, considerable importance was laid on the recycling of water (from atmospheric moisture), to be used by the cosmonauts for drinking and food preparation.

At the beginning of this mission, it was stressed by Major-General Georgii Beregovoi, Head of the Cosmonaut Training Programme, that it should not be considered simply as a preparation for the forthcoming Soyuz-Apollo project. Nevertheless, when the Soviet team for the project flew to Houston on February 8, for the next round of preparatory talks, there can be little doubt that both sides saw the success of the Salyut-4 mission as a promising augury for the joint venture.

Will the US make its own laws of the sea?

by Colin Norman, Washington

Two bills which are now being pushed independently in the United States Congress are likely to have a significant impact on the second session of the Law of the Sea Conference, which opens in Geneva next month. One measure is designed to protect the financial interests of American companies involved in mining operations on the deep sea bed, and the other would extend the jurisdiction of the United States over fishing rights from 12 to 200 miles. Both those issues were at the centre of intense debate during the first session of the Law of the Sea Conference held in Caracas last year, and the prospect of unilateral American action on either of them is certain to colour debate in Geneva.

As for the issue of mining on the sea bed, there was virtual deadlock in Caracas between developing nations, which generally supported the idea of establishing an international authority to regulate commercial operations and research on the sea bed, and industrialised nations, which were reluctant to give an international body so much authority over commercial enterprises. The importance of the bill which was introduced into Congress last week by Senator Lee Metcalf, a Democrat from Montana, is that it signifies that if the conference deadlocks again, the United States may well go ahead with regulation of deep sea mining on a unilateral basis.

Although Metcalf's bill was introduced last year and Congress failed to pass it, it should be regarded more seriously this time because Metcalf has made a deal with some powerful committee chairmen which will ease the bill's progress through the Congressional mill. The clear inference being given by Metcalf's staff is that if the Law of the Sea Conference deadlocks, the bill will be out of committee and on the floor of the Senate very quickly.

The impact of the bill on the Geneva conference should also be seen in the light of the recent claim on an area of the sea bed filed by Deepsea Ventures Inc., one of the leading companies involved in exploratory work on the mining of manganese nodules. Although the claim, which was filed in the United States in November last year, has not been recognised by the State Department, it signifies that Deepsea Ventures is prepared to press ahead with its mining operations no matter what happens at the Law of the Sea Conference.

Metcalf's bill, in short, would estab-

lish a licensing scheme, operated by the Department of Interior, which would issue exclusive licenses to American companies for prospecting rights on large blocks of the sea bed. Later, the companies would be able to apply for licences to begin commercial exploitation of minerals on the sea bed. Although the bill states that the licensing operation would be replaced by any mechanism that may be established by international treaty, the Administration has consistently opposed the measure on the grounds that it would pre-empt discussions at the Law of the Sea Conference.

Last year, the bill was passed by the Senate Interior Committee soon after the Law of the Sea Conference ended in Caracas, but the Senate Foreign Relations Committee claimed jurisdiction over the measure and kept it bottled up until Congress adjourned. This year, however, Metcalf has concluded a deal with the chairmen of both the Armed Services and the Foreign Relations committees which would give them only 30 days to consider the bill once it is passed by the Interior committee. At the end of that period, the bill would automatically go on the Senate calendar and be brought to a vote.

One other factor could influence the situation later this year. The Interior Department, which has consistently opposed Metcalf's bill, is now developing legislation of its own which will be introduced as an alternative measure if the Law of the Sea Conference is inconclusive. The bill, which was first brought to light by *Ocean Science News*, a newsletter well versed in the affairs of the marine science industry, would provide for prospecting licences to be issued to American corporations but would impose a moratorium on commercial exploitation.

As for the fisheries bill, a measure supported chiefly by Senator Warren Magnuson, a powerful Democrat from Washington State, was passed by the Senate late last year, but it was not considered by the House. Magnuson intends to re-introduce the measure this week and the House Committee on Merchant Marine and Fisheries will be holding public hearings during the second week of March on a similar measure.

The opposition of the Department of Defence was considered an important factor in preventing the bill from being passed by the full Congress last year, but there have recently been rumours—which the Department of Defense will neither confirm or deny—that the department no longer opposes the measure. If that is the case, Congress could well pass a fisheries protection bill while the Law of the Sea Conference is still in session. □

Fighting disease on the Volta River

from Peter Collins

THE development of new techniques for applying pesticides is being intensively studied by the Centre for Overseas Pest Research (COPR) as part of Britain's contribution to the vast onchocerciasis ('river blindness') control project now getting under way in the Volta River Basin. This project, sponsored by the World Bank, the UN Development Programme, and the World Health Organisation (WHO), is expected to cost some \$200 million over the next 20 years, with financial and technical cooperation from Canada, France, West Germany, the Netherlands and the USA, in addition to about £500,000 from this country as an initial contribution.

The opening phase of the project, managed by the WHO, aims to eliminate the black fly, *Simulium damnosum*, which is the vector of the parasite directly responsible for the disease, and which can only be effectively attacked during its larval stages. These are aquatic; the project thus involves a massive use of pesticides in the Volta rivers and their many tributaries in which the flies breed. It is here that the WHO has sought the help of the COPR, whose experience of tropical pest control was built up during the many years of its predecessor, the Anti-Locust Research Centre. In the present project, the problem is one of applying larvicides to many hundreds of miles of rivers running largely through dense forest, and of ensuring that they reach the larvae, which live in the fast-running stretches attached to sub-aqueous vegetation or to the river bottom, where they feed by filtering material brought down by the current.

Early trials, carried out by the COPR under contract to the WHO and in cooperation with a French onchocerciasis team, showed that the normal system of spraying from fixed-wing aircraft or helicopters was unsuitable or ineffective, partly because a large proportion of the pesticide is inevitably wasted on riverside vegetation. To avoid this, Cliff Lee, of the COPR, devised a method for dropping quantities of pesticides *en bloc* at a suitable distance upstream of the fast-flowing stretches favoured by the *Simulium* larvae. The prototype 'rapid release' device now being used in field trials is designed to apply accurately measured quantities of from 0 to 50 litres in a single drop, and has so far given extremely good results. The formulation devised for Abate (the pesticide chosen by the WHO as least likely to damage other aquatic species) is such

LAST month Madame Simone Weil, minister of health, declared that "the Pasteur Institute is no longer able to survive with only the help of its own resources," and deplored the fact that the prestigious institution "had been run by scientists who did not always possess the qualities of administrators."

Now the financial situation of the Institute is being examined by the *Cour des Comptes*, the government's accounting agency. When this report is completed, Madame Weil is to meet the institute's administrators and representatives of the scientific personnel in an attempt to find a solution for the Pasteur Institute "to continue its work in favourable conditions, either through increases in government subsidies, or by the State taking over some of its expenses."

The objective, says the dynamic and popular minister of health, "is to come to a point where the Pasteur does not have to keep asking for an amount of money it needs. A coherent and continuous system must be set up to allow the Institute to find its equilibrium." The Ministry of Industrial and Scientific Development, and that of Education, are also involved in the discussions.

This latest *crise* is one of many that have beset the Pasteur Institute since 1967, when a severe financial crisis triggered the so-called "Pasteur Institute revolution," which was followed by the replacement of a number of ageing administrators by scientists, and the drafting of new statutes providing for the separation of research and production activities.

As the new president of the institute's board of administrators, Jacques Monod attempted to maintain the Pasteur's status as a private institution (although one playing a vital national role) and to balance its books. He introduced into the hallowed walls of the building where Pasteur had carried out his experiments, a down-to-earth businessman, Jean Hardy, who came from the food industry.

Arrangements had to be made for some of the institute's products to be marketed by the pharmaceutical industry, and this gave rise to awkward situations. For instance, the Laboratoires Roger Bellon held a license to sell some of the Pasteur Institute's vaccines and biological products. Roger

Bellon was then purchased by Rhône-Poulenc, which also happens to have a controlling interest in the Institut Mérieux, which was founded by a disciple of Pasteur and is the Pasteur Institute's most successful commercial competitor.

Pasteur's progress

from Alexander Dorozynski, Paris



Monod: devoting himself to research

Two years ago, Institut Pasteur Productions, S.A. officially came into being, and took over commercial activities under the financial direction of Jean Hardy. Professor Monod agreed to head IPP temporarily as well as the Institute before returning entirely to scientific activities. It turned its profits over to the Pasteur Institute, but in spite of an annual production of some 17 million doses of serum and vaccines as well as of assorted biological testing products, and of substantial income from abroad from the licensing of the "prospective influenza vaccine" developed at Pasteur two years ago, ends wouldn't meet. The dream (which was defined by Professor Elie Wollman, associate director of the Institute, as that of a "socialist society of scientists, supported by a capitalist structure") just did not work out. The Pasteur got deeper into the red, and started eating up some of its capital, most of which came from the investment of donations of 2.5 million francs made at the time of the institute's founding in 1888 by contributors such as the Tsar of Russia, the Sultan of Turkey, and the Emperor of Brazil.

As a non-profit research organisation attempting to finance itself through a

commercial subsidiary, it remained in a difficult position. There is little doubt that if IPP had decided to go into outright competition with the profitable French pharmaceutical industry, chances of success should have been good. But marketing "Pasteur Institute Aspirin" or other current drugs under the Pasteur label was not in the tradition. "We are not a drug company," said Prof. Wollman. "We don't sell drugs and I don't expect we shall."

Towards the end of last year, Professor Monod proposed his radical "renovation plan": to move away from the original location of the institute in the city (abandoning the laboratories, the research hospital, the historical library, as well as the grave of Pasteur), sell the 50,000 square metres of valuable real estate for some 250 million francs, and move to Garches, a few miles north of Paris, where the institute has the use of a large estate and has some production facilities.

The plan was opposed by a vast majority of the research staff, led by François Jacob. Last month Professor Monod resigned his post as president of the directors "to devote himself fully to stimulating scientific research at the institute". He was replaced by "businessman" Jean Hardy, who remains at the same time director of Pasteur Productions.

It has been pointed out that Dr Monod's return to scientific activity has been planned long ago, but perhaps it was triggered by a feeling (shared by some members of the Ministry of Health) that an outstanding scientist is not necessarily a good administrator.

At the same time a department of corporate development was created, now headed by Joël de Rosnay, a scientist with several years' experience in industry.

The Pasteur's problem is not so much to survive—that it undoubtedly will—but to survive while keeping its independence from state control and *functionarization*. This independence has not paid off in cash terms, but it seems to have paid off in terms of research. If the awarding of Nobel prizes is a criterion of value, the argument is a strong one; out of nine Nobel prizes awarded to Frenchmen in the fields of medicine and biology, eight have been given to *pastoriens*. □

that it spreads rapidly on the surface of the stream, forming what has been described as "an almost monomolecular film". At the same time, it is found effective as far downstream as 50 kilometres from the point of release, which is some 200 metres above the first site to be dealt with in any one drop. This is in strong contrast to the range of the same material when

applied by orthodox spraying techniques some 15–80 kilometres downstream.

The prototype equipment, designed by the COPR and made by CIBA-Pilatus for the WHO, was intended for use with fixed-wing aircraft and the first trials were carried out with a normal light aircraft. It has now been adapted for use with the helicopters to which the WHO is at present com-

mitted on this project, partly because many of the rivers wind for miles through narrow walls of dense forest, where fixed-wing aircraft cannot safely be used. But on the larger and more open stretches, especially where sites to be treated are far apart, light aircraft may well prove more economical because of the much greater distances that they can cover. □

correspondence

Soviet dissenters in need of help

SIR,—The case of Vladimir Bukovsky (February 6) highlights a disturbing aspect involving my own profession. Bukovsky was released from a labour camp in 1970 after several spells of 'treatment' for his dissenting views. He spent the next 14 months of liberty collecting evidence of psychiatric abuses for the suppression of political dissent in the Soviet Union at great risk to himself. His only hope of protection lay in the forthcoming Congress of the World Psychiatric Association (WPA) in Mexico City in 1971. It was to psychiatrists in the West, and to the psychiatrists attending this congress in particular, that Bukovsky sent his covering letter in the form of an appeal when he sent the documents out to the West. Their authenticity has never been questioned. The Executive Committee of the World Psychiatric Association declined, however, to allow discussion of this problem despite the efforts of some psychiatrists. This shameful act of betrayal produced a savage reprisal in the form of a 12-year prison sentence and the terrible conditions under which Bukovsky has to exist. It is not a story that a psychiatrist can tell with pride.

We now have information that the Soviet authorities were extremely apprehensive about the Mexican Congress. At the time Mr Victor Fainberg, a dissenter, was in a Leningrad psychiatric prison hospital, from where he has now succeeded in emigrating to Israel. He tells me that he and his friend Borisov made certain demands for improving the conditions of the inmates of the hospital and for a re-examination of their own cases by the Court. Prior to the Mexico Congress conditions improved and the Soviet authorities agreed to have the cases re-examined. As soon as the decision of the Executive Committee of the World Psychiatric Association became known, conditions became worse than they had ever been and their request for re-examination by the Courts was dismissed with a contemptuous laugh. It can be assumed that in other special hospitals the same thing occurred. The failure of the WPA to rally to the defence of Bukovsky and the other 'lunatic' dissidents was an act of opportunism and devoid of all compassion.

I am writing to urge psychiatrists to do all they can to right a terrible wrong



Bukovsky: appealed to psychiatrists

that has been committed and to put all possible pressure, both individually and through their associations, on their Soviet colleagues and Soviet authorities to secure the release of a brave and generous man.

To move to the case of another dissident whose case has been mentioned in *Nature* recently, I can report that I have been asked by Mrs Plyushch to act as psychiatrist in an investigation into her husband's psychiatric state. This is in connection with legal action which she intends to bring against the Dnepropetrovsk special hospital for unlawful detention and criminal negligence in his treatment at that hospital.

Leonid Plyushch was sentenced to detention in a special hospital after examination by three psychiatric commissions, one of which pronounced him sane. The final one, presided over by Professor Snezhnevsky considered him as suffering from schizophrenia and stated that he had 'delusions of inventions', a reference presumably to Plyushch's interest in games theories, which he shares with his wife.

The case was heard *in camera* at Kiev and a large number of infringements of Soviet law appear to have been committed. Thus, defence counsel was not granted time for a proper interview and was told by the Judge to base his defence on evidence contained in the prosecutor's files.

A collection of Plyushch's letters has been published in Russian by the Herzen Press in Amsterdam. The first letter was written just after his arrest and the others after his incarceration in the Dnepropetrovsk special hospital, one of the most feared in the Soviet

Union. The letters show a lively, highly intelligent and understanding man for whom the experiences in the hospital were shattering. They show no indication whatsoever of mental illness.

According to information received only last week the 'treatment' received by Plyushch has reduced him to a state of complete nervous collapse so that proper psychiatric treatment under proper humane conditions might really be indicated at the present time.

I am prepared at any time to act as a psychiatrist representing the Plyushch family and would examine Mr Plyushch either at Dnepropetrovsk special hospital, or preferably for all concerned, in a hospital in the UK. Should Mrs Plyushch's application for emigration to the West for her husband, herself and two children be granted, I would be prepared to participate in any therapeutic measures that might be considered necessary to restore him to his previous good health.

G. A. LOW BEER

Consultant Psychiatrist,
Horton Hospital,
Epsom, UK

Hungarian visas

SIR,—On behalf of the Presidency of the Hungarian Biochemical Society and also in the name of those who made considerable efforts to ensure the successful organisation of the ninth FEBS Meeting in Budapest in 1974, allow me to express my profound disapproval of the letter entitled, "Entry forbidden" (December 13, 1974) that was written and sent to me as a New Year's present by Ms Peller.

I firmly believe that you are well aware of the fact that the Ninth FEBS Meeting was a success, and it served well not only the exchange of scientific information, but also international scientific cooperation and collaboration. May I quote a part from the letter of Professor H. R. V. Arnstein, Secretary-General of FEBS, addressed to Academician T. Erdely-Gruz, President of the Hungarian Academy of Sciences? "The organisation and scientific standard of the Budapest Meeting were excellent and I have heard many favourable comments. I am sure that the meeting will long be remembered with pleasure by all the biochemists who were fortunate enough to take part and it will have contributed significantly to scientific cooperation in Europe both now and in the future". Similar appre-

ciations were also received from a number of participants from all over the world.

It is incomprehensible to us why such real and true information about the ninth FEBS Congress did not appear in your journal. We think this would have indeed served international scientific relationships well.

Regarding Ms Peller's visa, the facts can be summarised briefly as follows. Ms Peller's request for a visa arrived in Budapest very late. In spite of this it was dealt with and the visa sent to Vienna, just as were a number of other visas of members of the Israeli Biochemical Society. Ms Peller arrived in Vienna a few days before her visa came through and she had not the patience to wait for it; the visa was left at the Hungarian Embassy in Vienna. I would like to mention that more than 2,000 active members from 38 countries participated in this congress and everybody who arranged their visa in due time was able to enter Hungary.

DANIEL BAGDY

*Secretary of the Hungarian
Biochemical Society*

Nutritional research

SIR,—The very real issue which concerns both John Rivers (January 10) and John Yudkin (January 31) in their recent correspondence on the Neuberger report is the balance which must be maintained between applied nutritional research into topics of human and social concern and the more basic type of research usually associated with nutritional biochemistry. There is a risk, however, that the intensity of their criticism might lead the non-nutritionist to believe that the policy makers, particularly those associated with the Medical Research Council (MRC), have shown little concern over this matter. This would be far from true and it is to correct this possible misconception that we are writing this letter.

In 1971 an MRC subcommittee, of which incidentally Professor Neuberger was a member, recommended a change in policy at the Dunn Nutritional Laboratory and this was subsequently approved by the council as follows: "The council recognise the need to lay foundations for more research designed to investigate specific nutritional problems both in the United Kingdom and overseas and they have agreed that in the future research programme of the laboratory there should be a change in emphasis from basic biochemistry towards applied nutritional studies, in particular clinical and epidemiological investigations. Biochemical research will continue in close relation with applied studies".

This policy statement became the basis of our present research pro-

gramme, all of which is related directly to a specific human or clinical problem. These studies have, however, to rely heavily on a firm backing of fundamental research, for in all too many nutritional disorders we lack the basic information to mount really effective applied programmes. Obesity is a good example. We just do not know why individual people lay down different amounts of fat on apparently similar energy intakes and expenditures. At the Dunn, obesity is being investigated on a broad front, using a whole-body calorimeter together with epidemiological studies into the functional significance of different degrees of obesity in terms of morbidity and exercise potential and metabolic studies on the relative economics of different enzyme pathways.

There is considerable interest concerning the role of dietary fibre and this, too, is the subject for a bivalent approach, defining more accurately the metabolic functions of the unavailable carbohydrates and quantifying, by epidemiological and clinical investigations, the practical benefits or disadvantages which might accrue from increasing the fibre content of the diet.

There is also concern about the nutritional status of elderly people, especially those living alone. Recent evidence has suggested the possible existence of sub-clinical riboflavin and vitamin C deficiencies, but in our present state of knowledge we do not know the real significance of the findings. Again, we have an epidemiological investigation under way to reveal the environmental and sociological factors which are causing these abnormalities, linked with laboratory research to define what they mean. Only in this way can we plan effective action should this prove necessary.

Bone disorders are also a problem of the aged and the role of vitamin D in these is likewise under intensive study.

These are just a few of the community-oriented research projects in the UK and overseas in which we are involved and in which we are trying to achieve the same sort of scientific balance.

It is perhaps unfortunate that on first appraisal the Neuberger report does seem to contain more on biochemistry than on 'social' nutrition, but closer scrutiny will show that it quite specifically states that there is a need for more research in this area as well as on other aspects of human and clinical nutrition. The positive point which comes out of the report and the criticisms it has invoked is that nutritional science is very much alive and of importance, not just in an international context but for the health and welfare of people in the UK as well. Nutrition has to be a broadly based science and

it is up to nutritional scientists to make certain their subject is able to develop along balanced lines. This is what the debate should be all about.

R. G. WHITEHEAD

W. P. T. JAMES

*Dunn Nutritional Laboratory,
Cambridge, UK*

SIR,—As a nutritionist who worked for more than 10 years in the Nutrition Division of the Food and Agriculture Organisation of the United Nations, I feel I must take issue with several statements in the Joint ARC/MRC Report on Food and Nutrition Research.

I strongly support those who have already expressed the view that the Report is excessively biased towards the cellular and subcellular level of nutrient activity, and virtually ignores the fact that nutrition is concerned primarily with the food that people eat. The opportunity has been missed for stressing the inseparable interrelationships between agriculture and nutrition, both quantitatively and qualitatively. Thus one question that urgently needs answering is whether this country can increase its food production beyond the present level that meets only about half of our consumption, and in particular how in doing so it can make the most useful contribution to human nutrition? Such a possibility of collaboration between the two Research Councils is not mentioned.

I am one of those nutritionists who have long believed that, however much research still needs to be done in such areas as the effects of nutrients on metabolism, we already know enough to seek to apply our present knowledge to large scale improvements in the health in our own country and in the rest of the world. But to do this, we need a far more aggressive attack on the problem of how to affect people's eating habits than the lukewarm attitude indicated in the report by such statements as: "On the evidence of published literature, the effect of advertising on food consumption patterns appears not to have been studied," or "It may also be desirable to find ways of changing patterns of food consumption when supplies are limited." Do we need another war, or a series of world food crises, to persuade us that this is a problem crying out for research?

In considering training for research in nutrition, the Committee has clearly not surveyed the facts, for it says that it considers that first degrees in nutrition and food science "make relatively little contribution to research potential." This is simply not true. In my work with FAO, I have for example met graduates in nutrition from Queen Elizabeth College carrying out research

in places as far apart as Ottawa and Accra, Kampala and Colombo, Kuala Lumpur and Freetown. Their research was being done at universities, institutes of agriculture and medicine, and government research laboratories, and was in subjects over a wide range, from anti-oxidants and synergists to infant weaning practices and diets. My own experience suggests that a higher proportion of nutrition graduates are actively engaged in food and nutrition research than graduates from the biomedical fields which the Committee has suggested would be more appropriate.

In the section on training for research in nutrition, there is however one useful remark, that the necessary characteristic of the nutrition specialist is that he should have a broad outlook, and that his function is to catalyse and summarise both teaching and research. Were these not the essential features in the department created by John Yudkin when he was appointed as Professor to the first school of nutrition in this country devoted to undergraduate and postgraduate teaching and research at Queen Elizabeth College at the University of London? Professor Yudkin's attitude towards nutrition was reflected in his teaching colleagues, his research students and is now shared by hundreds of nutrition graduates, working in a wide variety of fields, in many countries round the world.

It would be invidious for anyone to criticise the composition of the Committee, possessing as it did such a wealth of knowledge and experience, or to question the esteem of the co-opted members of its Working Parties. Nevertheless it remains true that only three of the fourteen members of the Committee could consider themselves as expert in human nutrition. Even more extraordinarily, neither the Committee nor the forty or more members of the Working Parties had a single representative from either of the two schools which give first degrees in nutrition and which have made many notable contributions to research over the broad field of nutrition.

We in the Polytechnic at Huddersfield do not accept the views implicit in what must come to be known as the Neuberger Report. We are planning to introduce a course for the degree of MSc in Social Nutrition within a few years which will have as one of its major objectives the training of graduates from food science, nutrition, catering studies and dietetics in the social concomitants of nutrition as a preliminary to preparing them for research in this field.

Yours faithfully,
T. B. MORGAN
*The Polytechnic,
Huddersfield, UK*

Early tunnellers

SIR,—When I discussed early British tunnelling projects (Jan. 31, 1975) a printer's imp substituted "early 1880s" for "early 1800s". In fact Trevithick nearly completed his Limehouse-Rotherhithe tunnel under the Thames soon after Waterloo and the Brunels succeeded in the 1840s. This will be found to help the sense which contrasts the preoccupation with tunnelling at the beginning of the 19th century with the switch to bridging towards the end.

ANGELA CROOME

London SE3, UK

Food from allotments

SIR,—With commendable humility, the World Food Conference in Rome called on science and research workers to bridge the gap between the incompatible elements of its action pack (*Nature*, 252, 518; 1974) and I hope I have disentangled the metaphor correctly).

May I, then, suggest a tactic not considered in Rome—one which would pay off within two years, unlike the global meteorological networks, the intensified research, the remote sensing or the testing of new technology in farmer's fields? I merely suggest that everyone who can, especially in the 'have' countries, feeds himself.

It seems plain to me that hunger cannot be alleviated by shifting money about the world, but only by growing more food. Britain survived two wars on the produce of her allotments (over half the island's food was grown on them). If all who have consciences will devote 150 hours a year to a garden, they will do some good, unlike those who walk a few miles on one day of the year to collect money.

I had better explain the word 'allotment' for an international readership. In several countries, municipalities supply, at a nominal rent, small gardens (allotments) to interested citizens: "A plot of 300 square yards" (say 1/40th hectare) "should supply a family of three all year round" (*Encyclopaedia Britannica*, 1951, article Allotment).

There is nothing in this scheme to attract the notice of the Starvation Industry—no intercontinental jet flights, no twenty-thousand-dollar-a-year jobs, no lush research grants. So we common people must demand that land be set aside for allotments in every town. Then each of us can solve a tiny part of the problem. No more can be expected of us.

How big is the problem? I gather we need 45 million extra rations. If, then, 90 million of us—thirty million families—grow half our own food, the immediate problem is solved. With vigorous promotion, there could certainly be 30 million allotments more in the world within two years. It is no dream to pre-

dict 300 million allotments, world-wide, within five years, if we try.

Those who have no land should demand it, where practical, from their local authorities.

F. P. HUGHES

Ontario, Canada

Vial body wanted?

SIR,—It is now more than ever necessary to minimise waste in laboratories, as elsewhere. This seems to make it imperative to devise means, possibly centralised regionally or nationally, for saving and reusing vials for liquid scintillation counting. It seems that, up to the present time, almost all such vials have been discarded and destroyed after being used once only. The numbers of vials so used and destroyed annually in Britain must be very large. In this department alone, the number last year was about 100,000.

Arguments against reusing vials are well known; the main ones are that: (1) the vials may have been irreparably damaged; (2) the vials may be irreversibly contaminated; (3) arrangements for retaining, decontaminating and washing used vials may endanger the environment and particularly, of course, the health of personnel; and (4) such arrangements are uneconomical. These arguments are valid. There are, however, counter-arguments, which seem to have been made unanswerable by the increasingly adverse economic and environmental conditions in which we find ourselves. The essential argument is that the destruction of once-used vials is unacceptably wasteful as the cost of producing them rises and as the materials from which they are made become shorter in supply.

I should like to propose, therefore, that a committee representing vial users, including university, research council and industrial laboratories, be set up to investigate urgently the practicability and economics of the centralised collection, storage and preparation of used vials for reutilisation. There should be little difficulty in collecting the information required for establishing quite rapidly whether this can be done economically on a regional basis. Individual laboratories should find no insuperable difficulties in collecting, storing and despatching used vials safely and efficiently.

If those who agree with this proposal and are prepared in principle to act on it would write to me, the response will be communicated to the research councils and other official organisations in the hope that action will be initiated.

Yours faithfully,

G. V. R. BORN
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news and views

The programming of the visual cortex

by Miranda Robertson

IN the past five years the organisation of the mammalian visual cortex has become the focus of a nature-nurture controversy whose claim to more than usual interest lies in its definition at the level of single neurones and their connections in the brain. The neurological foundations of the debate have been laid very largely by Dr David Hubel and Dr Torsten Wiesel, and the relative absence of dispute over their picture of the neurophysiological basis of vision is almost more surprising than the disagreement over whether, as they think, it is innate, or, as many others insist, it is subject to substantial environmental modification in early life.

The essence of the original description of adult cat cortex (Hubel and Wiesel, *J. Physiol., Lond.*, **160**, 106-154; 1962 and **165**, 559-568; 1963) is thus unchallenged. Cells in the primary visual cortex (area 17) respond to simple line stimuli such as light slits, edges and dark bars. The nature and selectivity of the responses vary with depth from the cortical surface in a way that reflects the specific connectivity of the cells, which Hubel and Wiesel have classified as 'simple', 'complex' and 'hypercomplex'. The functional subdivision of the cortex into layers of simple and more complex cells has since been correlated with the anatomical division into layers of stellate and pyramidal cells (van Essen and Kelly, *Nature*, **241**, 403-405; 1973). At right angles to the layers, the cortex is divided into columns of cells whose responses are tuned to lines of a specific orientation.

In more recent work, Hubel and Wiesel have extended the principle of columnar organisation in work on the distribution of ocular dominance in the monkey, which has revealed alternating sheets of cells fed predominantly by right and by left eyes (*J. Physiol., Lond.*, **195**, 215-243; 1968). They have now gone on to examine more precisely the columnar geometry of orientation specificity and its relationship with ocular dominance. In their three most recent papers they present an arresting picture of the 'functional architecture' of areas 17 and 18 of the monkey cortex, hinting at its implications for the genetic specification of neuronal

organisation and remarking on the influence of environmental factors only for its apparent absence.

The first of the three papers (*J. Comp. Neurol.*, **158**, 267-294; 1974) is concerned with quantitative and morphological definition of the ocular dominance and orientation columns. Hubel and Wiesel now see the columnar organisation as a device for mapping ocular dominance and orientation specificity (and probably other variables, such as colour, which they did not investigate) systematically on to a three-dimensional space whose axes are already engaged with the topographic representation of the visual field (for the surface coordinates) and complexity of processing (for the depth coordinate). From this and their second paper, which is concerned with receptive field size, they have inferred a structure of crystalline regularity, composed of myriad identical columnar machines, each equipped for the complete analysis of a portion of the visual field equivalent in size to the receptive fields of the individual neurones of a column, plus their scatter. They have described the fundamental analytical unit as a hypercolumn—a functional aggregate comprising a left-right pair of ocular dominance columns and a set of orientation columns containing all the specificities in a 180° cycle.

The measurements which led to this inference were made by advancing a microelectrode 20-50 µm at a time tangentially through the cortex, recording the ocular dominance and orientation specificity of each cell encountered and marking the electrode track for later histological examination. Hubel and Wiesel found that ocular dominance alternates regularly at intervals of about 0.25-0.5 mm, and orientation preference changes regularly clockwise or anti-clockwise, with occasional abrupt reversals, completing a cycle within about 0.5-1 mm. Orientation columns seem, like ocular dominance columns, to be sheets rather than cylinders, although the resolution of electrophysiological techniques is so far inadequate either to establish this precisely or to determine whether the changes in orientation preference are discrete or continuous. Nor is the relationship between ocular dominance and

orientation columns clear, although the two systems seem to be independent.

The hypercolumn emerged from a comparison of the sizes of orientation and ocular dominance columns, which differ by an order of magnitude. This struck Hubel and Wiesel as odd until they realised that a right-left ocular dominance pair is about the same width as a complete 180° cycle of orientation preferences: fuse the two and you have a hypercolumn.

The dimensions of the orientation columns have further functional implications. Neuroanatomical studies of cortical cells have shown that the dendritic arborisation of pyramidal cells extends well over the width of an orientation column. This supports the suggestion that the fine tuning of orientation-selective neurones is mediated by lateral inhibition—a postulate for which there is already neuropharmacological evidence (Pettigrew and Daniels, *Science*, **182**, 81-82; 1973; Rose and Blakemore, *Nature*, **249**, 375; 1974).

The second paper is concerned with the uniformity of the hypercolumnar machinery across area 17 of the cortex. The density of the retinal projection to the striate cortex is far from uniform: the density of retinal cells and thus of visual input decreases with eccentricity from the fovea. This is reflected in an increase in the receptive field size of cortical neurones with eccentricity of retinal projection. It is not, however, reflected in any variation in cortical cell density or columnar width: foveal and peripheral information seems to be processed identically.

It is the last of the three papers that contains the basis for contention, for it is here that Hubel and Wiesel describe the extension of the investigations of the preceding two to young monkeys without visual experience. They used four animals. Two whose eyelids were sutured within two days of birth, and one whose eyelids were sutured immediately on delivery by caesarian section, were examined after a few weeks. In one monkey, recordings were made two days after birth.

From data on some 300 cells in all, including 23 in the normal 2-day old, they conclude that the structure they have described for adult cortex is complete and fully functional from birth.

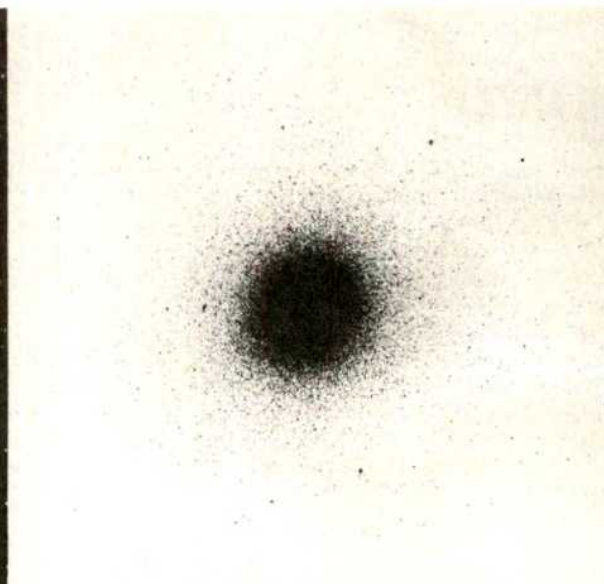
They did detect minor deviations from the normal pattern: a few cells lacked clear orientation specificity, and an abnormally low proportion of neurones responded to binocular stimuli. This is attributed to the early effects of visual deprivation, which is the only environmental factor whose influence they acknowledge. Both ocular dominance and orientation specificity can be disrupted by depriving the cortex of input; but the modification involved represents a non-adaptive deterioration in the existing cortical organisation.

This is precisely the position Hubel and Wiesel adopted on the cat ten years ago, and which has been called into question by the later experiments of Barlow, Blakemore and Pettigrew (Barlow and Pettigrew, *J. Physiol.*,

Lond., **218**, 98–100P; 1971 and Blakemore and van Sluyters, *J. Physiol.*, *Lond.*, **237**, 195–216; 1974), who claim that neither orientation specificity nor ocular dominance is fully determined in newborn kittens, and that both develop in the first weeks of life under the influence of visual input. Hubel and Wiesel now concede, in the light of this and neuroanatomical evidence, that the kitten cortex is immature at birth. Monkey cortex, however, is anatomically mature and, it now seems, neurophysiologically indistinguishable from that of the adult; since its columnar organisation is also in all known respects closely similar to that of the adult cat, Hubel and Wiesel argue that the development of adult properties in cat neurones is a function

of maturation and not of experience.

How is this view of a genetically programmed, structurally uniform, functionally stereotyped repeating crystal of miniature machines to be reconciled with the mass of evidence accumulated over the past five years by (most notably) Blakemore, Pettigrew, and Mitchell, for profound and permanent modification of the orientation specificity and binocular connections of cortical cells by early experience? Numerous experiments on kittens reared in exclusively spotted, vertically striped, or horizontally striped worlds have demonstrated that the majority of cells in the cat visual cortex respond preferentially only to orientations which they have experienced. Blakemore and Pettigrew (Blakemore and van Sluy-



*Some of the spectacular photographs already obtained with the 3.9 m Anglo-Australian Telescope at Siding Spring. Left, two exposures of NGC 2442, a barred spiral galaxy with asymmetrical arms and a bright nucleus. Right, two objects in our Galaxy: top, the giant globular cluster 47 Tucanae which is only 14,000 light years away and contains some 10^6 stars; bottom, Eta Carinae, a large hydrogen emission nebula with wide dust trails and narrow lanes ('elephant trunks') where cold interstellar matter is squeezing into the hot gas. Eta Carinae's central star may be a new one, as yet unstable.

ters, *J. Physiol., Lond.*, **237**, 195–216; 1974 and Pettigrew, *ibid.*, 49–74) propose that immature cells can respond to a wide range of orientations which is narrowed as a consequence of experience, probably through the strengthening of inhibitory intracortical connections which 'tune' the response curves of the complex and hypercomplex cells. Blakemore and van Sluysers have gone further, and suggest that orientation preference is innately biased in the simple cells, which transmit the bias to the immature complex and hypercomplex cells of a given column and thus provide an innate substrate for the regular repetition observed by Hubel and Wiesel. But in their visually deprived monkeys, Hubel and Wiesel found representatives not only of simple cells, but of complex and hypercomplex cells with adult properties.

Blakemore and van Sluysers insist that visual experience actually modifies cortical connections, and does not simply cause selective deterioration: there are no silent areas. For example, monocular suturing of kittens' eyes reversed during the plastic period of development (up to 14 weeks) causes a reversal of the cortical ocular dominance pattern.

For aspects of the nature–nurture conflict on which both factions have experimental evidence, the best hope of a resolution in favour of plasticity lies in the experimental procedures used by Hubel and Wiesel. There was at one time a suggestion that Hubel and Wiesel had mistakenly recorded as orientation detectors cells which were in fact merely movement detectors, which are relatively crude entities characteristic of immature cortex. They have now, however, introduced controls which go some way towards answering these criticisms. The other question which has been raised is the completeness of the early visual deprivation. Evidence has recently been presented that cortical modification can take place within as little as an hour's experience (Pettigrew, Olson and Barlow, *Science*, **180**, 1202–1203; 1973). The speed and effectiveness of the suturing of the eyelids of newborn monkeys (whose cortex everyone agrees is anatomically mature) thus becomes critical.

Otherwise, the only way of reconciling the conflicting observations is by the intuitively unappealing compromise of a cortex whose elaborate and fully functional innate organisation can be substantially respecified as a result of experience. This is horribly unpar-simonious because if cortical plasticity does not take the onus of functional adaptation off the genome it is hard to see its evolutionary advantage in a visual world which as far as we

know has been fairly constant.

The question of the evolutionary significance of cortical plasticity has been specifically addressed by Blakemore and van Sluysers, who believe that its crucial function may be the matching of orientation specificity for the inputs of both eyes to the binocular cells of the cortex. Binocular cells as defined by Barlow *et al.* (*J. Physiol., Lond.*, **193**, 327–342; 1967) have an essential property that has not been examined by Hubel and Wiesel in their binocularly driven cells. They represent the neurological substrate of depth perception and are acutely sensitive to disparities in the position of the image on the two retinæ, which vary as a function of distance. Clearly the input to a binocular cell must be tuned to precisely the same orientation for both eyes. Blakemore and van Sluysers concur with Hubel and Wiesel in finding a few (20%) cells with binocular connections in the absence of visual experience. They have shown, however,

that without patterned stimulation the orientation preference of the two inputs is poorly matched. Hubel and Wiesel have not reported on the matching of inputs to the binocular cells in visually inexperienced monkeys.

Thus, much of the contention devolves upon how much is still not known about cortical function. It is now agreed that at least a part of the structural basis for visual function is laid down at birth; the question is whether it is then modified and refined by experience. This question cannot even be definitively posed while it is still unclear how much is not known about the visual cortex. It has been estimated that neurophysiological techniques sample something like 50 cells in a population of about 2,500 encountered by an electrode in the course of a single penetration, and there is no guarantee whatever that the sample is representative. It is thus surprising that the lines of dispute can be drawn so clearly on a basis of accepted data.

Palaeomagnetic field matters

from Peter J. Smith

ONE of the most fascinating phenomena in the history of the earth sciences is the way in which palaeomagnetic research and the arguments about continental drift developed in parallel for over 40 years, coexisting within the scientific world but never really meeting. Possible reasons for this intellectual failure are not difficult to find in retrospect. For one thing, as a reading of Koenigsberger's classic reviews shows (*Terr. Magn. atmos. Elect.*, **43**, 119 and 299; 1938), interest in the magnetism of rocks (going back well into the eighteenth century) was largely a concern for what would now be termed rock magnetism rather than palaeomagnetism—the solid state physics and mineralogy of magnetic grains as opposed to any relevance the magnetism might have to the history of the Earth. As such, it was a subject to be pursued by physicists rather than geologists and thus liable to fall victim to the growing separation of the sciences and increasingly difficult communication. Wegener's hope that his *Die Entstehung der Kontinente und Ozeane* (1915) would re-establish the connection between geophysics on the one hand and geology and geography on the other was not to be fulfilled for many a year.

The second, perhaps more important, reason for the early failure to connect palaeomagnetism with the continental drift debate was that the few genuine palaeomagnetists about had something quite different in mind. As late as 1949, for example, Graham (*J. geophys. Res.*, **54**, 131; 1949) could head his objectives

with the claim that "if this pattern of primary magnetisation [in sedimentary rocks] could be shown to be permanent . . . it should be possible then to determine how the direction of the Earth's magnetic field has varied during geological time". The previous year, Johnson *et al.* (*Terr. Magn. atmos. Elect.*, **53**, 349; 1948) had made their primary aim even more explicit: "In order to determine the origin and nature of the Earth's magnetic field and to test the various hypotheses which have been advanced to explain the field, it is desirable to determine the history of this field throughout geological time and to investigate more carefully its spatial variations, both inside and outside the Earth's surface." Their own work on Pacific sediments and the glacial clays of New England was one of the first important steps in this direction.

When the palaeomagnetic method and the hypothesis of drifting continents did finally come together in the late 1950s the mixture was explosive. The combination also changed the palaeomagnetic ground rules as they would have been understood by Johnson and his colleagues. For one thing, the growing evidence for drift made it clear to the pre-revolution palaeomagnetists that they were to be thwarted in one of their primary tasks—that of using the magnetism of rocks to test directly whether or not the geomagnetic field had always been essentially dipolar. From now on the dipole hypothesis would become a

Necessary assumption (not without considerable justice) rather than an idea to be confirmed or refuted. Perhaps more significantly, the aims of palaeomagnetism were expanded to cover not just the relatively narrow geophysical problem of tracing the geomagnetic field through time but also the much wider geological problem of delineating the movements of land masses. Or to put it another way, the wider geological community had begun to discover the Earth's magnetic field. But in bringing the larger and smaller communities together it was inevitable that henceforward the geological aspects of the subject should dominate the geophysical ones.

Nevertheless, fundamental work on the origin and long-term behaviour of the geomagnetic field continued throughout the earth science revolution, albeit on a relatively small scale; and some of it (most notably the construction of the geomagnetic polarity-time scale) even found important geological application in its own right. In recent times, however, there has been a noticeable upsurge of interest in the study of the palaeomagnetic field for its own sake, almost 20 reports having been published in the last six months alone. And it is apparent from the diversity of subjects covered that the detailed delineation of the history of the Earth's magnetic field is still far from complete; even the comfortably familiar is not beyond question.

The article by Verosub on page 707 of this issue of *Nature* offers a good case in point. Since the pioneering work of Elsasser (*Phys. Rev.*, **69**, 106; 1946 and **70**, 202; 1946) in the mid-1940s, the self-reversing dynamo has come to be accepted as the only viable source of the main dipole field. There are problems in establishing the validity of the dynamo model because, although mechanical analogues can reproduce field reversals and field intensity fluctuations, they are a poor substitute for the complex motions thought to occur in the Earth's core; and the motions themselves have not so far proved amenable to satisfactory mathematical treatment. Nevertheless, the self-reversing dynamo has reigned supreme if only because every other idea has been ruled out as impossible. Or so we thought. Verosub, however, offers a delightfully simple (in principle) alternative involving two opposing fields whose magnitudes vary with time. This model poses its own problems, of course—most notably the need to find the origin of the second field (the first presumably being produced by a non-self-reversing dynamo in the fluid outer core). But by the same token, it also brings the whole question of field origin back within the scope of non-magnetohydrodynamic man.

In support of his two-component field source hypothesis Verosub quotes Wilson's (*Geophys. J.*, **28**, 295; 1972) discovery that there are significant differences between the time-averaged mean pole positions of normal and reversed populations—differences which Verosub believes might indicate the dominance of a distinct field source in each polarity state. Using Upper Tertiary and Quaternary palaeomagnetic data from

the Soviet Union, Wilson showed that whereas both normal and reversed poles tend to lie on the far side of the present geographical pole from the observing site, the 'far-sidedness' in the reversed case is significantly greater than in the normal. Earlier, Wilson (*Geophys. J.*, **19**, 417; 1970) has interpreted far-sided poles in terms of an axial dipole displaced a small distance northwards from the centre of the Earth; and this is a theme to which

Time to ecological equilibrium

Responses from Leigh Van Valen, T. R. E. Southwood and Donald Strong

STRONG (*Proc. natn. Acad. Sci. U.S.A.*, **71**, 2766; 1974) has pointed out flaws in the correlation shown by Southwood (*J. Anim. Ecol.*, **30**, 1; 1961) between subfossil records of trees and the associated insect species richness. He showed that Southwood's correlation was confusing by demonstrating a correlation between the present range of British trees and the number of associated insect species. He also estimated that the time required for tree genera introduced into Britain to become saturated with herbivores was only a few decades or hundreds of years—the evidence for this being that the regression of herbivores on the distribution of non-native trees does not differ significantly from that of native genera. Peter D. Moore discussed his findings in *Nature* (**252**, 14; 1974).

● Leigh Van Valen now writes that Strong's last conclusion is unjustified because "analysis of data from Strong's Fig. 3 shows that the slope of regression for introduced genera not only does not differ significantly from that for native genera, it also does not differ significantly from either 0 or 1, whether or not two ambiguous genera (apple and lime) are included. Therefore the conclusion, while quite possibly true, is not supported by the data."

● Peter Moore, who expressed in his article in *Nature* reservations about Strong's evidence against an increase in species richness over geological time, now adds, "I agree that one cannot justify a rapid attainment of saturation by insect species on the basis of Strong's data. On the contrary, one might predict that if any non-native tree species in Britain were to extend its range significantly then a rise in associated insect species might be expected. The question of changes in richness during geological time remains unsettled."

● T. R. E. Southwood emphasises

that although he is responsible for the correlation between geological time and species richness, in his original paper he also pointed out the relationship between the present range of trees and their associated species richness (see *Proc. Hawaiian ent. Soc.*, **17**, 299; 1960; *XI Int. Kongress für Ent.*, **1**, 651–655; 1961 and *Nature*, **253**, 313; 1975).

● Donald Strong replies: "Van Valen is wrong. Given the correlation coefficient values characteristic of host plant–insect species richness relationships, one does not expect high significance values for comparisons of few points; my conclusions are not contingent upon the significance of the correlation of introduced points. Since the original publication, however, I have been supplied range data on two additional introduced hosts, walnut and spruce (through the great courtesy of Dr Perring's unit at Monk's Wood). With these included, the correlation coefficient of introduced taxa is significantly greater than 0 ($r=0.635$; $0.05 < P < 0.1$). These data will be included in a forthcoming paper dealing with other pest species over these same hosts. This new paper will demonstrate rapid species saturation as does the first. As well, I have found rapid saturation in a totally different plant–insect system (*Science*, **185**, 1064–1066; 1974).

"Southwood's comment is correct, and Moore is confused, as reference to my original paper will verify. One of my main theses was that pest species richness will vary as host range varies, quickly, be the species introduced or native. My alternative to the non-asymptotic model was clearly stated to be an asymptotic one."

"Please note also that there is an error in my original paper; in Fig. 3 'common maple' sits not to the left of the regression line as I have plotted it, but virtually on top of it. This correction reinforces my conclusions."

he and McElhinny (*Geophys J*, **39**, 571, 1974) have now returned. An up-to-date analysis of 291 worldwide late Tertiary and Quaternary palaeomagnetic pole positions confirms the phenomenon of far-sidedness and also suggests that poles tend to lie to the right of the geographical pole when seen from the observing site. The revised figure for the average northward offset of the dipole for the past 25 million years is 325 ± 57 km (standard error), but there are now enough data available for them to be divided into smaller sets. The dipole offsets for the periods 25–7, 7–2 and 2–0 million years are 555 km, 316 km and 143 km, respectively, showing that the axial dipole has been moving gradually towards the geocentre.

On this occasion, Wilson and McElhinny did not consider the normal and reversed data separately, so it is not yet possible to say whether the time-averaged differential offset indicated by the Soviet results is valid on a worldwide basis or not. Looking at a shorter time scale, however, the whole question of polarity states and reversals continues to exercise the apparatus of the mind and the laboratory in a variety of ways. Aldridge and Jacobs (*J geophys Res*, **79**, 4944, 1974), for example, have constructed mortality curves for each normal and reversed phase of the Earth's magnetic field over the past 45 million years and find that they differ from a simple exponential distribution for the lifetime of the phases. It is possible, of course, that the distribution should not be exponential anyway. But Aldridge and Jacobs consider it more likely that the observed departures from an exponential form arise from short polarity events that have not yet been detected—a conclusion similar to that reached, albeit in different ways, by several others beginning with Cox (*J geophys Res*, **73**, 3247, 1968).

The ratio of the number of real polarity phases to the number of those actually observed so far is apparently about 2. This indicates an appreciable number of reversals remaining to be discovered, and may go a long way towards explaining the prevailing confusion over the identification of short polarity events. As Noel and Tarling point out on page 705 of this issue of *Nature*, for example, the so-called Laschamp event has been detected in at least 12 localities at reported ages ranging from 7,000 to 17,000 years. But are all the claims for the recognition of the Laschamp valid, or are there really two or more events involved here? And what is an 'event' anyway? Some years ago a polarity event was defined as a short period ($<10^5$ years) of opposing polarity within a much longer polarity epoch. But McElhinny (*Palaeomagnetism and Plate*

Tectonics, Cambridge University Press, 1973) has suggested that brief events such as the Laschamp may not be true reversals at all but only 'excursions' of the geomagnetic field—relatively short-term deviations of the palaeomagnetic pole beyond that reasonably attributable to normal secular variation but something less than a full 180° reversal. Indeed, some workers, such as Freed and Healy (*Earth planet Sci Lett*, **24**, 99, 1974), have already taken to referring to the 'Laschamp excursion'.

But how do field excursions arise? (They are known to occur, irrespective of whether or not the Laschamp event is one of them.) Possible causes are a tipping of the dipole towards the equator or a reduction in dipole strength which would allow the non-dipole field components to predominate. The latter is perhaps the more likely, and in this connection it may be significant that Aldridge and Jacobs mention dipole strength as possibilities for their missing events, that Cande and Labreque (*Nature*, **247**, 26, 1974) offer field intensity fluctuations as a possible explanation of the small scale magnetic lineations recorded over the North Pacific floor by deep tow magnetometers, and that Greenwalt and Taylor (*J Geophys Res*, **79**, 4401, 1974) claim that low magnetisation blocks detected in the mid-Atlantic ridge "could represent actual periods when oceanic crust was generated during a time of significantly reduced main field strength". In short, there are clear suggestions that dipole fluctuations may account for several phenomena which are otherwise difficult to explain.

Which brings matters to one of the most intractable of all palaeomagnetic problems—that of how to determine the strength of the ancient geomagnetic field and plot its variation, if any, with time. There are two main difficulties here. The first is that although a palaeomagnetic direction may be obtained from a rock just as long as a measurable amount of the primary magnetisation remains, a palaeomagnetic field intensity may generally only be determined if the proportion of primary magnetisation remaining is known. It seldom is, although ways are available of mitigating the problem in certain cases. The second difficulty is that the obvious ways of obtaining a palaeofield from a rock containing thermoremanent magnetisation require that the rock be heated above its Curie point, a process likely to alter the magnetic minerals either physically or chemically. Clearly it would be desirable to have a method of determining ancient field intensities which could either be applied at room temperature or, if heating is necessary, would enable any alteration in the rock to be avoided or estimated. Curiously, three possible



A hundred years ago

THE fitting of the Arctic ships *Alert* and *Discovery* is making rapid progress at Portsmouth, in the hands of the dockyard shipwrights, who are working extra hours, in order that they may be rigged and out of their hands by the 12th of April. The sledges have all been made, and the tents are in progress. Meanwhile the officers are pursuing their special studies. We understand that Commander Markham, and Lieutenants Archer, Giffard and Fulford are going through a course of instruction in magnetism. Lieutenants Parr and May are to be initiated into some special astronomical work, and two other lieutenants will receive charge of the pendulum observations. The work connected with spectrum analysis will also be provided for, and one or more of the officers will take up photography. The ships will be commissioned in the middle of April, and will sail early in June.
from *Nature*, **11**, 355, March 4, 1875

candidates have appeared 'almost simultaneously, all of them involving anhysteretic remanent magnetisation (ARM). The most satisfactory appears to be Shaw's (*Geophys J*, **39**, 133, 1974), a method which does not eliminate heating but which permits selection of a coercive force region within which the heating has not changed the magnetic properties. Banerjee's method (*Earth planet Sci Lett*, **23**, 177, 1974) requires a single heating but is claimed to be preferable to those methods requiring repeated heatings, and the technique described by Stephenson and Collinson (*Earth planet Sci Lett*, **23**, 220, 1974) involves no direct heating but requires the assumption that parameters relating to some rocks are also valid for others. No method is completely problem-free.

Finally, it must be said that this discussion of recent work on the palaeomagnetic field is far from exhaustive. During the past few months Georgi (*Geophys J*, **39**, 71, 1974) has presented a spherical harmonic analysis of palaeomagnetic inclination data, Baag and Helsley (*J geophys Res*, **79**, 4918 and 4923, 1974) have constructed a new geomagnetic secular variation model and carried out a shape analysis of palaeosecular variation data, Creer (*Earth planet Sci Lett*, **23**, 34, 1974) has determined geomagnetic variation recorded in a Black Sea sediment core 7,000–25,000 years old and so on. Statistical anomaly or new commitment?

Solar influences on the weather

from R H Olson

A symposium on solar-weather relationships sponsored jointly by the American Meteorological Society and the Solar Physics Branch of the American Astronomical Society was held in Denver on January 23

THE solar-weather symposium was designed to focus the attention of both the meteorological and astronomical communities on this perplexing problem. Primary emphasis was not on reporting of new empirical findings, but on physical concepts. For example, J Toomre of the University of Colorado compared the problems of describing the convective activity on the Earth and on the Sun. In spite of the difference of many orders of magnitude in the Rayleigh and Prandtl numbers on the two spheres, some similarities exist. A mathematical technique borrowed from meteorology has been found useful by solar physicists in describing solar convection. It is based on the anelastic equations, which assume a quasi-compressible fluid. In similar vein, P Gilman of the National Center for Atmospheric Research (NCAR) compared the horizontal circulation patterns of the Earth and Sun. He pointed out the great importance of eddy components in driving both circulations. The importance of convection on the Sun as opposed to solenoidal fields on the Earth was mentioned in describing the energy sources for the circulation. Perhaps the greatest unsolved problem in the solar circulation is to explain the increase in rotation speed toward the equator (that is, the equatorial acceleration).

Empirical findings were not entirely neglected. J Eddy of NCAR gave a historical review of the field. He reminded the audience of a period of almost complete absence of sunspots which occurred from about 1645 to 1715. This 'Maunder minimum' was discovered in the early years of the century, but has been largely forgotten. It is an intriguing thought that this greatest known anomaly in the behaviour of the Sun comes in the middle of the greatest climatological event in recent centuries, namely the 'Little Ice Age', a period of abnormally low temperatures and precipitation.

Other statistical relationships were pointed out by J King of the Appleton Laboratory (Slough). He gave examples of 11-year and 22-year periodicities in weather phenomena. But R Shapiro of Air Force Cambridge Research

Laboratories pointed out that such apparent cycles are difficult to prove.

Getting around to the physical side of solar-weather relationships, the cupboard is still fairly bare. There are some interesting possibilities, however. R Dickinson of NCAR suggested that the only physical process known to have a large solar cycle modulation and reaching the troposphere is Galactic cosmic ray ionisation. This ionisation may nucleate sulphuric acid aerosol, which in turn could provide efficient cloud condensation nuclei, thus modulating cirrus cloud cover at high altitudes and latitudes. He mentioned recent findings that solar flares cause large and sudden increases in NO_x species in the upper atmosphere, which can lead to large scale destruction of ozone.

A Dessler of Rice University summed up possible mechanisms which might bear investigation. One which has not been widely discussed before is the leakage of electric current from the auroral region into the lower atmosphere. He also developed the theme touched on again and again by Eddy and other speakers, namely that our knowledge of the possible variations in the so-called solar constant is abysmally poor. Variations of anywhere from 0.1% to 0.5% or more could easily go undetected with our present observing capabilities. Even if the lower figure is examined, it is obvious that the energy available from possible variations in the solar constant is 1,000 times the energy available from the magnetosphere, as far as influencing the atmosphere goes.

The problem of the lack of knowledge of the solar constant also provided perhaps the closest thing to a clash of opinions. D Williams of the National Oceanic and Atmospheric Administration suggested that in view of the gross uncertainties in this parameter it was pointless to pursue solar-weather studies until this area is cleared up. W Roberts of the University of Colorado retorted sharply that he was not willing to wait five years or longer to go on looking into the important problem of solar-weather relationships. Perhaps some progress in measuring the solar constant with high precision is in the offing. D Heath of NASA Goddard mentioned that Nimbus satellites to be launched in 1975 and 1978 are scheduled to repeat from space the surface-based measurements made by Drummond and others of the solar constant. Other satellites will attempt to improve the global monitoring of climatic elements, such as ocean environment, atmospheric heat budget, and trace elements. Global maps of precipitation are being made now by satellite, and will soon be available for use.

The great importance of the Soviet efforts in solar-weather studies was at least touched upon, although one had the feeling that only the tip of this particular iceberg was showing. V Mikhnevich of the Applied Geophysical Institute, Moscow, reported on attempts to derive mathematical-physical models to explain the propagation of disturbances, caused by solar activity, in the upper atmosphere down to the lower atmosphere. G Gromova of the Hydrometeorological Centre, Moscow, reported on a study of the kinetic energy budget of the atmosphere, averaged over latitudinal zones. An increase in kinetic energy three days after magnetic storms was contrasted with reduced kinetic energy following geomagnetically quiet times.

King suggested that atmospheric modelling is needed to set the framework for solar-weather relationships. J Wallace of the University of Washington and NCAR replied that before useful models could be built a definitive pattern of observed relationships is needed. C Leith of NCAR remarked that current models of short-term atmospheric changes have serious shortcomings in their ability to explain atmospheric changes. Thus there is ample room for the models to include such additional inputs as solar influences if any can be shown to exist.

The Earth as a radio source

from A P Willmore

AMONG the planets, we are accustomed to think of only Jupiter as a powerful radio source, but results obtained recently with the satellites Imp 6 and Imp 8 in interplanetary space show that the peak radio emission from the Earth exceeds that from Jupiter by nearly two orders. The experiments, described by Gurnett (*J Geophys Res*, **79**, 4227, 1974), were designed to study plasma waves propagating in the magnetosphere and interplanetary space, the geocentric distance of Imp 6 ranging from 6,613 to over 200,000 km, while that of Imp 8 ranged from 150,000 km to 300,000 km. The spacecraft were each equipped with long dipole aeriels of lengths approximately 50-100 m and with three mutually orthogonal loop aeriels, so that both the electric and the magnetic field components of the waves could be measured. The aeriels were coupled to sensitive wide-band amplifiers whose output was analysed by multichannel spectrum analysers covering the spectral range 36 Hz to 178 kHz, extended up to 2.0 MHz by a tunable receiver in the case of Imp 8.

At distances of several Earth radii, Imp 6 detected sporadic bursts of noise

in its upper frequency channels, above about 30 kHz. The duration of the bursts ranged from 0.5 to several hours, with intervening intervals of as much as 24 hours during which the signals might be undetectable. The first problem was to identify the type of wave being detected. On comparing the results from the two sets of aerials, it was found that the electric and magnetic field intensities were linearly related, with a constant of proportionality which identified the waves as ordinary electromagnetic waves. The frequency spectrum of the bursts was determined with Imp 8 and shown to be remarkably narrow, most of the

radio wave energy being contained between 60 and 300 kHz.

When Imp 8 was at a distance of about 200,000 km from the Earth, the measured electric field signals were found to be strongly spin modulated, showing that the waves originated from an approximately point source, since had their distribution been isotropic the signal would not have depended on the orientation of the spacecraft. In fact the source was shown to have an angular diameter of less than 12° and to be in the direction of the Earth. At this distance, the diameter of the Earth subtends 4° , so though the Earth was clearly the source, its precise location

on the Earth could not be determined. On the other hand, a source relatively low in the atmosphere, rather than in the magnetosphere, is suggested, a supposition which is rather strengthened by the observation that, statistically, the intensity of the bursts falls off in an inverse square law fashion with distance from the Earth, at least at considerable distances (more than 4 Earth radii).

More precise information on the location of the source, however, was obtained from the intensity variations at various points in the magnetosphere. The waves were not observed whenever the spacecraft were inside the boundary surface known as the plasmapause

LONGO and Penhoet reported¹ that a rat glioma releases, among other molecules, a protein which shares some immunological, chemical and biological properties with the mouse salivary nerve growth factor (NGF). A correspondent writing in these columns² about their work raised the question of a possible new role for the glial cell. Though this question is legitimate on theoretical grounds I wish to point out other findings which would not support the hypothesis.

NGF is present in large quantities in snake venom and male mouse submaxillary glands, but these are not the only sources of NGF. This protein molecule is also released from some mouse sarcomas³ and is produced by granuloma⁴ and embryonic tissues⁵. Recently a sensitive immunoassay was used to show that two neoplastic cell lines, L and 3T3, derived respectively from mouse C3H subcutaneous and adipose tissues and from simian virus-40 transformed A31, likewise produce a biologically active NGF which is immunologically similar if not identical to mouse submaxillary gland NGF⁶. Since in the experiments by Longo and Penhoet the NGF-like protein was isolated from solid rat tumours which were obviously contaminated with fibroblasts and other host cells, one cannot decide whether the NGF was of glial or fibroblastic derivation, and one wonders why the investigators did not extract the NGF protein from a pure glial cell line rather than from transplanted tumours.

In favour of an NGF-releasing role of glial cells the correspondent also quotes some recent experiments by Swedish investigators who reported that NGF injected intracerebrally enhances regenerative processes in noradrenergic nerve cells in the CNS⁷. Since glial cells outnumber nerve cells in the CNS

A new role for the glial cell?

a reply from Rita Levi-Montalcini

in the ratio of 10:1, these findings seem to the correspondent to suggest that this astronomically large cell population may provide the NGF which could not readily enter the brain from the bloodstream. It should however be remembered that the number of noradrenergic nerve cells in the CNS is of the order of a few thousand (the locus coeruleus, by far the largest noradrenergic nucleus in vertebrate brains, contains about 1,400 noradrenergic neurones⁸) while the whole neuronal population in the mammalian CNS is in the range 10^8 – 10^{10} according to the size and phylogenetic position of brains. Hence only one out of a million nerve cells in the CNS would be receptive to NGF. An NGF-releasing role of glial cells would therefore benefit only an exceedingly small nerve cell population.

The situation in the peripheral nervous system is not much better. Regeneration of damaged axons occurs in peripheral nerves, irrespective of the receptivity of their cells of origin to NGF. Somatic motor and sensory differentiated neurones do not in fact respond to NGF and yet their axons are tightly wrapped by glial cells. Furthermore sympathetic neurones show a maximal NGF response at an early stage of differentiation when only a few glial and other satellite cells are present in the ganglia and are very loosely scattered among, but not adherent to post-ganglionic axons.

The possibility that glial cells surrounding other nerve cell types might contain hitherto undiscovered nerve growth factors cannot be discarded. But before considering this

it is highly desirable to obtain much more convincing evidence for the release of NGF by cells unequivocally identified as glial cells.

The correspondent suggests using immunofluorescence histochemistry to see whether NGF can be found in glial cells which are "wrapped around" their target neurones. But glial cells are not wrapped around noradrenergic neurones in the vertebrate CNS. The locus coeruleus consists of a small population of densely packed nerve cells in reciprocal contact with each other. Glial cells are not seen around individual neurones. Only a few loosely scattered, small non-neuronal cells are found intermingled with nerve cells of this nucleus, and there is no way of deciding whether these are glial cells or other cell types. Since some fibroblastic lines release NGF (refs 4–6) a positive fluorescence reaction would still not prove that glial cells release NGF. Even if they do, the NGF is probably present in the cells that produce it in such small quantities as to be undetectable by this technique.

While therefore there is no *a priori* reason to object to an "NGF-releasing role" of glial cells in the same way as this property has already been proved for a number of other cell lines, the hypothesis submitted by the correspondent that this role would explain the intimate relationship between glial cells and neurones is considerably weakened by the above considerations.

¹ Longo, A. M., and Penhoet, E. E., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 2347–2349 (1974).

² *Nature*, **251**, 100–101 (1974).

³ Levi-Montalcini, R., and Hamburger, V., *J. exp. Zool.*, **116**, 321–362 (1951).

⁴ Levi-Montalcini, R., and Angeletti, P. U., in *Proc. 4th Int. Neurochem. Symp.* (edit. by Kety, S. S., and Elkes, J.), 362–376 (Pergamon Press, New York).

⁵ Bueker, E. D., Schenkman, I., and Bane, J. L., *Cancer Res.*, **20**, 1220–1228 (1960).

⁶ Oger, J., Arnason, B. G. W., Fantazis, N., Lechrich, J., and Young, M., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 1554–1558 (1974).

⁷ Bjorklund, A., and Stenevi, U., *Science*, **175**, 1251–1253 (1972).

⁸ Descarries, L., and Saucier, G., *Brain Res.*, **37**, 310–316 (1972).

This divides the charged particle plasma which corotates with the Earth (in fact the upward extension of the ionosphere) from that which is fixed in an Earth-Sun coordinate system due to the existence of the solar wind. That the waves were not observed within the plasmopause is quite natural, since the ambient density of charged particles is too high for the propagation of electromagnetic waves of low frequencies. The fact, however, that a shadow zone could be traced in the magnetosphere out to distances of 15 Earth radii, showed that the waves must originate in the auroral zones of the atmosphere. Finally, correlation of the observation of particular bursts of radio noise with observations of the auroral regions by the USAF Dapp satellite, which obtains a picture of the auroral oval, showed the electromagnetic radiation to originate in discrete auroral arcs, generally in the evening portion of the auroral zones. Evidently the waves are generated by the intense fluxes of energetic electrons which produce the auroral arcs.

The production process must be efficient, since the energy associated with the electrons is typically 10^{11} W, whereas that in the radio bursts is 10^9 W. Certainly some cooperative mechanism or plasma instability is required, which is true also in the case of the radio emission from Jupiter. There are other similarities with Jupiter, among others large fluxes of energetic electrons moving along the magnetic field lines are involved in each case, and the frequency of emission also appears to be similar to the electron cyclotron frequency in the emitting region. Gurnett concludes that the types of instability proposed to account for Jovian emissions may also account for the terrestrial radiation.

Catecholamine receptors detected

from Leslie L. Iversen

THE current vogue for labelling receptor sites has resulted in spectacular progress towards identifying sites for various peptide hormones, neurotransmitters such as acetylcholine and glycine, and drugs, including morphine (see Dismukes, *Nature*, **252**, 442, 1974). Catecholamine receptors, however, have proved more elusive. Various attempts to identify β -adrenoceptors in tissues such as liver, heart or spleen by measuring the binding of labelled catecholamines or their antagonists to intact cells or to microsomal fractions failed to demonstrate a binding process with the expected properties. Cuatrecasas *et al.* (*Nature*, **247**, 92) reviewed their own experience in this field at the

Microwave spectra of molecular ions

from our Chemical Physics Correspondent

MICROWAVE or rotational spectra of neutral molecules are straightforward to study, but the difficulties multiply for short-lived molecular ions in the gas phase. This is unfortunate since interstellar conditions, with a comparative absence of collisions, are much more favourable to long life for such species. Indeed the structure HCO^+ has been tentatively suggested for "X-ogen".

Further interest arises since Bunker (*Chem Phys Lett*, **27**, 322, 1974) has pointed out that species such as HD^+ and $^{14}\text{N}^{15}\text{N}^+$ should have quite strong microwave spectra. The corresponding neutral molecules have zero dipole moment, unless extremely small effects are considered, and consequently they have no detectable microwave spectrum. For charged species the dipole moment is no longer independent of the origin of the coordinates to which it is referred. Although if this origin is at the centre of the ion the dipole moment will be equally small, for discussing microwave spectral intensity, as Bunker shows, the appropriate origin is the centre of mass so that species with different isotopes may have quite strong spectra.

Similarly they have significant infrared absorption intensity. In HD^+ the calculations suggest a dipole of 2.9×10^{-30} C m (0.87 D) and a vibrational transition moment of 2.8×10^{-31} C m (0.086 D).

Alongside this theoretical feature one must place the experimental success in the laboratory of Dixon and Woods (*Phys Rev Lett*, **34**, 61, 1975) in the detection of a spectrum due to CO^+ . A pair of lines at 117 692.55 and 118 101.99 MHz due to $J 1/2-1/2$ and $1/2-3/2$ respectively were observed. Special techniques with a sample length of 3.5 m included the discharge to form the ions and a signal averager to enhance the low signal-to-noise ratio through repetitive scanning. Because of the unpaired electron the spectrum is sensitive to magnetic fields and the detection of both lines enables the spin-rotation constant γ_0 to be evaluated as 272.96 MHz and the rotational constant B_0 as 58 983.13 MHz. Although moderately accurate values were previously known from optical spectroscopy, these more precise values should be an excellent guide to radioastronomers who wish to seek for CO^+ .

beginning of 1974 and persuasively demonstrated that the binding of ^3H -noradrenaline to such preparations detected a site that recognises primarily the catechol function of the molecule. This site, unlike the β -adrenoceptor, does not distinguish between the active and inactive stereoisomers of the catecholamines or their antagonists and may be a form of the enzyme catechol-O-methyl transferase.

A great deal of advice and criticism flowed during 1974 as to how the job should be done, and it is perhaps not surprising that by the end of the year success should finally have been attained in the search for a method of labelling catecholamine receptors. Indeed, three groups have now reported binding assays which exhibit the characteristics predicted for β -adrenoceptors. Their success in the face of many previous disappointments illustrates the critical importance of choosing the right ligand and the right tissue for receptor binding studies. The three groups, Levitski *et al.* (*Proc natn Acad Sci USA*, **71**, 2773 and 4246, 1974), Lefkowitz *et al.* (*Biochem Biophys Res Commun*, **60**, 703, 1974) and Aurbach *et al.* (*Science*, **186**, 1223, 1974) all used high specific activity β -adrenoceptor antagonists as ligands, the compounds

being DL- ^3H -propranolol, L- ^3H -alprenolol and ^{125}I -hydroxybenzylpindolol respectively. All of these drugs are very potent antagonists at β -adrenoceptors, with binding constants of the order of 10^9 l mol $^{-1}$. In addition all three groups used the membranes of avian or amphibian red blood cells as the source tissues for binding studies. Such cells are known to possess a catecholamine-sensitive adenylate cyclase activity that is triggered by a β -adrenoceptor mechanism, although the physiological significance of such receptors in red blood cells remains unclear.

The binding of the labelled antagonists to the cell membranes was found to be saturable and a considerable proportion of the binding (as much as 80% in the case of Lefkowitz *et al.*) could be prevented by the addition of an excess of non-labelled propranolol or other known antagonists. Furthermore, the active L-stereoisomers of the antagonist drugs propranolol and alprenolol were more than one hundred times as potent as the inactive D-isomers in preventing binding of the labelled material. Binding could also be inhibited by other β -adrenoceptor antagonists, and by catecholamines such as isoprenaline, noradrenaline, adrenaline or dopamine, but not by α -adrenoceptor antagonists such as phen-

tolamine, or by neutral or acidic catechol substances. Isoprenaline was more potent than adrenaline or noradrenaline and dopamine was only weakly active, in keeping with the known potencies of these substances as agonists at β -adrenoceptors. The effects of the catecholamines were also stereospecific, with the biologically active L-isomers being about one hundred times more effective in preventing ligand binding than the inactive D-isomers.

Dissociation constants for the antagonist drugs in the binding assays were in the nanomolar range and were similar to those determined in these and earlier experiments for these substances as antagonists of catecholamine-stimulated adenylate cyclase activity in the erythrocyte membrane preparations. Dissociation constants for the catecholamine agonists were about two orders of magnitude higher and were again similar for the binding assay and the adenylate cyclase effects. The apparent affinities of various antagonists and agonists for the binding sites thus paralleled the biological effectiveness of these compounds on the β -adrenoceptor stimulated adenylate cyclase, a finding which lends strong support to the conclusion that the binding assays are indeed specifically detecting β -adrenoceptors. The number of such receptor sites on each turkey erythrocyte is approximately 1,000 (Levitski *et al.*) and corresponds to 0.2 pmol mg⁻¹ membrane protein in frog erythrocytes (Lefkowitz *et al.*), values that are much closer to estimates of receptor density for peptide hormones in fat or liver cell membranes than the much higher figures previously obtained in catecholamine binding studies.

Although the present reports represent the first successful identification of catecholamine receptors, it remains to be seen whether this approach can be extended to β -adrenoceptors in more complex tissues. Other catecholamine receptor sites, such as α -adrenoceptors or dopamine receptors in CNS still, of course, represent unconquered peaks

Estonian wetlands

from Peter D Moore

WETLAND habitats have proved particularly sensitive to the agricultural and industrial demands of man in the last two centuries. Those which have not been drained for agriculture or exploited for peat reserves have often been polluted or eutrophicated by a variety of effluents. Much international effort has been aimed recently at the conservation of wetlands, both because of their innate vulnerability and because of their immense biological

interest. Popular concern, directed chiefly towards spectacular waterfowl, has not proved difficult to stimulate.

Information on the current state of affairs in the Estonian Soviet Socialist Republic is now internationally available as a result of the recent publication in English of their 7th contribution to the International Biological Program entitled *Estonian Wetlands and their Life* (Academy of Sciences of the Estonian SSR, 1974). This collection of papers is concerned with the status of wetland habitats and wetland species in Estonia, it provides some welcome information on the conservation measures currently being invoked for their protection and on some of the fundamental scientific work in progress on the wetland reserves.

Estonia, with an area of 45,215 km², has about 1,150 lakes, covering 2,130 km² (4.8% of the country's area) and about 18,000 km² (20%) of peatlands, and some of these areas are of international importance for wildlife conservation. Conservationists in Estonia, particularly Professors E. Kumari and V. Masing, have proposed to the IUCN that twelve areas be recognised as worthy of high priority for conservation. These cover a total area of almost 90,000 ha, and four of the sites (about 28,000 ha) are already state nature reserves.

The Matsalu Bay area on the west coast of Estonia is a well-known site of ornithological interest, where greylag goose, bittern and marsh harrier breed. The mute swan, which also breeds here and is prized as a rarity (about 60 pairs in Estonia), can hardly be regarded as worthy of international interest. Matsalu Bay was raised to the status of a state nature reserve in 1957 by the direct action of the Supreme Soviet of the Estonian SSR, although wildfowling had officially been banned in the area since 1947. Despite this there are still problems, such as the influence of land management in neighbouring state and collective farms, recreational boating and sporting activities.

In a country so rich in peatlands, it is natural that these should figure prominently among the sites of conservation value. Six peatland sites, comprising over 30,000 ha are proposed for conservation, only one of which, the Nigula peat bog in south-west Estonia, is currently a state nature reserve. Many of these peat bogs have suffered less interference at the hands of man than almost any other Estonian habitat and their vegetation and structure are of particular interest. About 30% of Estonian peatlands are of an ombrotrophic type, the remainder being fens and transition mires. The ombrotrophic mires are largely raised bogs of the continental type, often be-

ing wooded with stunted pines. As one moves from the western bogs towards the east, so oceanic species such as *Myrica gale* give way to continental dwarf shrubs like *Chamaedaphne calyculata*.

The bird life of the Estonian peat bogs adds to their biological importance. Such species as wood sandpiper, whimbrel and great grey shrike breed in some numbers on these bogs, but the crane, willow grouse and peregrine falcon have declined very severely during this century, particularly during the last two decades. Between 1951 and 1961 the nests of the peregrine falcons, which were often situated on the ground in peat bogs of the Baltic region, were frequently found to contain only one egg, previously two eggs had been normal. By 1960, 80% of the breeding population had been lost and it is now considered extinct as a breeding bird in Estonia. Broken and added eggs were found during the days of the peregrine's decline, but no mention is made in this account by Kumari of the probable involvement of pesticides in this process.

The medium is the message

from Robert Shields

PROBABLY the most widely studied phenomenon in cell culture is that of contact inhibition of division. This occurs when normal cells are grown until they form a layer a single cell thick over the surface of the culture vessel, at which point (the saturation density), cell division decreases dramatically and the cells are said to be contact inhibited. Contact inhibition has come to be the *sine qua non* of normal cells, as tumour cells will often grow long after contact has been established and to far higher saturation densities—indeed the saturation density achieved *in vitro* is directly related to their tumorigenicity *in vivo* (Aaronson and Todaro, *Science*, **162**, 1024, 1968).

The role of cell-cell contact *per se* in the development of contact inhibition has been in doubt for some time, since many normal cell lines can be persuaded to reach higher saturation densities by more frequent changes of the culture medium or by increasing the concentration of serum in the cultures. These results suggested that the culture medium might be the limiting factor and that exhaustion of serum might be especially crucial. This was conclusively demonstrated by Dulbecco when he showed that the saturation densities achieved by the mouse 3T3 fibroblasts (but not the epithelial lines tested) was not related to the surface area of the culture vessel (as would be expected if cell growth was limited by cell-cell

contact) but rather to the supply of medium (*Nature*, **246**, 197, 1973). These experiments implied that cells stop growing when the medium is exhausted. But this does not explain two important observations. The first is that medium removed from contact-inhibited cultures will support the growth of non-confluent cells, showing that the medium is not exhausted. Second, if a strip of cells is removed from a confluent contact-inhibited culture, the cells at the edge of the 'wound' and those that migrate into the denuded area will divide, while the cells in the confluent cell layer which share the same culture medium will not. The 'wound' experiment was seen as the most persuasive evidence that cell-cell contact itself plays a part in contact inhibition and that contact somehow desensitises the cells to growth-promoting factors in the medium.

How does this contact-induced desensitisation occur? A possibility originally proposed by Rubin and expounded by Stoker (*Nature*, **246**, 200, 1973) is that there is a layer of medium in close contact with the cells which does not exchange freely with the bulk of the culture medium. Access of the cell layer to medium components would be limited by diffusion across this boundary layer and this limitation would lead to contact inhibition.

The diffusional flux of medium solutes across this boundary layer may be increased in two ways. One is to increase their concentration in the bulk of the medium (which would explain why the addition of increased concentrations of serum will stimulate growth in contact-inhibited cells), the second is to decrease the depth of the diffusion boundary layer. This vanishes at the edge of the cell sheet (which explains why cells in the wound can divide while those in the cell layer do not). Also the depth of the layer can be decreased by increasing the velocity of medium flow over the cell sheet: a fourfold increase in velocity will double the diffusional flux across the boundary layer—effectively doubling the concentration of medium factors reaching the cell surface (Maroudas, *Cell*, **3**, 217, 1974). This interpretation of the mechanism of contact inhibition was supported when Stoker showed that pumping medium rapidly across a confluent cell sheet will promote some cell division along the medium stream. In a more recent paper Stoker shows that the vast majority of contact-inhibited cells can be induced to divide without changing the medium (*Cell*, **3**, 207, 1974) if the velocity of medium flow across a cell layer is greatly increased by vigorous shaking of the culture.

The question that remains is what are the crucial medium factors needed by the cell to promote cell division?

This depends on the cell type and the conditions of culture but in cases where the bulk of the medium is not depleted, diminished access to serum factors across the boundary layer is probably the cause of contact inhibition. The fact that many tumour cells do not exhibit contact inhibition may be ascribed to their relative insensitivity to depletion of serum in the culture medium, so that even at high cell densities diffusion across the boundary layer is sufficient to provide the small amounts of serum necessary for tumour cell growth. If this was the case then tumour cells selected for inability to grow in low concentrations of serum should become contact inhibited. This has been shown to be the case (Vogel and Pollack, *J cell comp Physiol*, **82**, 189, 1973).

So it seems that contact inhibition (at least in mouse fibroblasts) may be explained by the existence of a diffusion boundary layer that prevents ready access to serum factors in the culture medium rather than by cell-cell contact. Whether serum sensitivity will replace contact inhibition as the new criterion for the normality of cells remains to be seen.

Terns as predators

from our *Animal Ecology* Correspondent

THE classic model of predator-prey interaction proposed by Volterra (*Mem Acad naz Lincei* (ser 6), **2**, 31, 1926) has been criticised on many grounds. Most criticisms have centred on the biologically impossible constraints concerning the reproductive rates of both predator and prey and their movements in time and space. In real life the dynamics of interactions are influenced by many factors such as a sharply defined breeding season and a time lag between the consumption of prey and the production of young as a response to it by the predator.

Maynard Smith and Slatkin have drawn attention to the likelihood that stability in predator-prey interactions is strongly influenced by yet another factor, namely the variety of hunting abilities normally found in mixed-age populations of predators (*Ecology*, **54**, 384, 1973). According to their calculations stability is maintained by the conservation of a prey species brought about by the strongly selective effects of the density of both it and its predator, and the differential in hunting ability found within the predator population. There is little doubt that the young of some species are totally ineffective hunters until they have been taught all the finer points of stalking. Remarkably little is known about the ecological effects on the prey species of such a life history strategy even with respect to the best documented species.

It is interesting, therefore, to note the work of Buckley and Buckley on the feeding ecology of the royal tern, *Sterna maxima* (*Ecology*, **55**, 1053, 1974).

Working in the Netherlands Antilles islands where royal terns spend the winter, Buckley and Buckley recorded data on several aspects of feeding behaviour. First, they observed as many dives as practicable and noted whether or not they resulted in fish being caught. If fish were dropped after being caught, this too was recorded. Second, they recorded the time budget of birds by counting the numbers of adults and juveniles on the roosts. Subtraction of this from the total number gave a measure of the time spent fishing. Third, they recorded the length of the periods when adults were fishing alone, juveniles fished alone, or both age classes together. Their results showed that adults' foraging passes lasted about half as long as did those of juveniles. Adults made 1.7 times more dives per minute as juveniles and the number of fish eaten per minute was 1.6 times that of juveniles. This was not because juveniles obtained fewer fish per dive than adults—although they frequently dropped, but recaptured, the fish—but that their diving rate was lower. These observations are consistent with the notion that fish are difficult to catch but that an increased fishing rate can offset this difficulty. The relative absence of adult terns, but not juveniles, from areas low in fish suggests further that adults are more adept at detecting prey than juveniles, and so work to a more relaxed time budget.

Some years ago Salt and Willard attempted a component analysis of predation of Forster's tern but did not, unfortunately, compare adult with juvenile fishing success (*Ecology*, **52**, 989, 1971). They suggested that searching rate by birds is inversely proportional to prey density. In the light of the Buckleys' study this should be modified to include 'within age classes'. The drop in attack rate in early spring noted by Salt and Willard was probably the result of the sudden influx of juveniles into the population and the rise of successful captures from spring through to winter an expression of a change in the structure of the prey population.

The royal tern study has demonstrated clearly that several foraging variables are subject to change with respect to age of individual and associated age structure of the predator population. Little by little reliable data are emerging which show that the accepted axioms of the Volterra approach are in need of revision. As far as terns are concerned, the cheeping of tiny beaks may be a blessing in disguise to later population stability.

articles

Differentiation of the Skaergaard Intrusion

Alexander R. McBirney

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Re-examination of the Skaergaard intrusion of East Greenland has revealed that the model of differentiation based on an original liquid with the composition of the chilled margin and a large hidden zone is no longer tenable. The composition of the Skaergaard magma has been determined experimentally for various stages of its evolution, and these compositions are consistent with revised volumetric and compositional data for the individual units of the intrusion. In its later stages, the magma separated into two immiscible liquids, one rich in iron and phosphorus and the other rich in silica and alkalis.

THE Skaergaard intrusion of East Greenland is a prime example of an igneous body that differentiated to an extreme degree through crystal fractionation. There are probably more geological and geochemical data on the intrusion than on any other igneous body in the world, but for some time it has been recognised that there are several puzzling aspects of these data that conflict with the inferred form and pattern of differentiation of the Skaergaard magma. The fractionation scheme deduced by Wager¹ in 1960 from mass-balance calculations required a large Hidden Zone amounting to about 70% of the total volume and extending almost to the mantle. The shape and composition of this postulated Hidden Zone were unlike those of any other known igneous body and seemed to violate certain mechanical and petrological principles. In 1971, another study of the intrusion was initiated in the hope that new geophysical and petrochemical techniques might provide the added insight needed to resolve some of these questions, and expeditions to the Skaergaard region were made in 1971 and 1974. The results of some of this work have already been published^{2,3} but most is still in progress. Here I describe some salient findings of an experimental study of high temperature phase relations and their bearing on the differentiation mechanism of the Skaergaard magma.

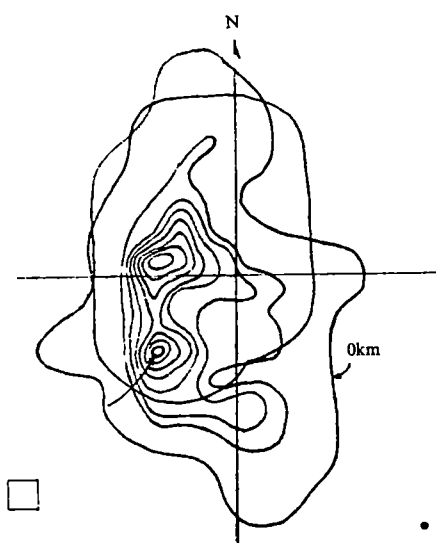
Measuring initial composition

The intrusion provides a wealth of information and is probably the only differentiated gabbroic body for which there are enough data to determine with any degree of confidence the composition and conditions of crystallisation of the evolving magma. The record of differentiation is well displayed in a 2,500-m sequence of layered gabbros. Because these rocks were formed from precipitated mineral assemblages that differed in composition from their parent liquid, there is no direct way of determining the composition of the differentiating magma. The method used by Wager¹ was based on a presumed initial composition which he took to be that of an analysed specimen of the chilled margin. Successive residual liquids were calculated by subtracting from this original composition the rocks of the Layered Series in amounts proportional to their stratigraphic thickness. The various factors used in this calculation were subject to serious errors, and the resulting calculations, though the best available, were open to question⁴.

Wager's model required a large Hidden Zone to balance the observed Layered Series and make the total intrusion have a bulk composition equivalent to the chilled margin. Gravity and magnetic surveys⁵ have produced little evidence to support that hypothesis (Fig 1). Furthermore, new analytical data on samples of the chilled margin¹⁸ show that the composition used by Wager as the basis of his calculations was in some respects unrepresentative (Fig 2), the same is true of the compositions of the Layered Series and Border Groups for which Wager was forced to use averages that in some cases were based on a single analysis.

Recent theoretical and experimental advances have made it possible to estimate parental liquid compositions directly from the rocks themselves. One can estimate the conditions of crystallisation from the nature of precipitated mineral assemblages and then determine experimentally the composition of liquids that are in equilibrium with these assemblages under the appropriate conditions. The Skaergaard rocks are well suited for such an approach, as there is already considerable data on the conditions of crystallisation. Temperature and oxygen fugacities for the Layered Series have been calculated⁶ from the coexisting iron-bearing phases, and the temperatures and total pressure at the last stages of crystallisation were determined from the relations of polymorphs of SiO_2 and $\text{CaFeSi}_2\text{O}_6$ (ref 7 and Nash, unpublished). These data show that total pressure was about 500 bar at the Sandwich Horizon and about 1,200 bar at the base of Lower Zone A. During the present investigation an experimental study of the Lower

Fig 1 Subsurface structure of the Skaergaard intrusion calculated from gravity and magnetic surveys⁵ assuming a density contrast between the gabbro and gneiss of 0.3. Contour interval is 500 m, maximum depth (arrowed) is 3.84 km, b square in bottom left is 1 km on a side.



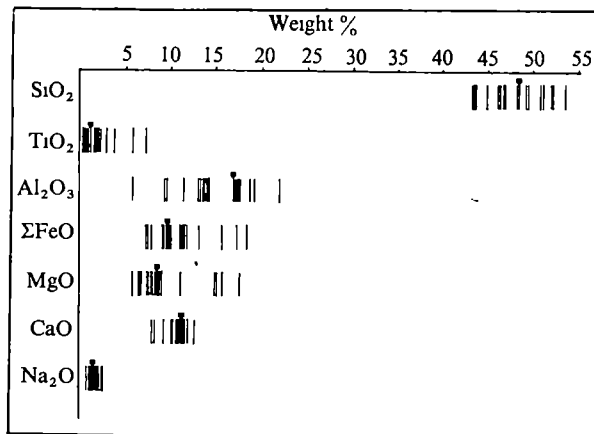


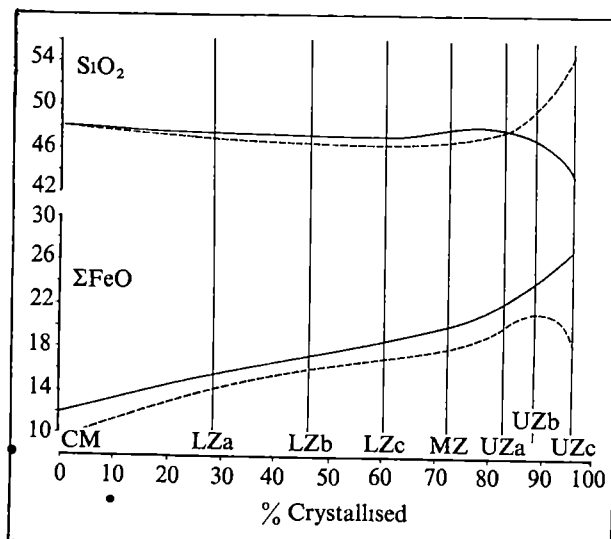
Fig 2 Variations in the major element compositions of analysed samples of the chilled margin. Values used by Wager¹ in his calculations, shown by the small arrows, are close to the mean of the new data but do not necessarily correspond to the composition of the original liquid. Some of the observed variations result from contamination effects of the gneissic wall rocks, others probably reflect deuteric effects, including transfer of mobile components in both directions after the chilled margin was formed. It now seems doubtful whether any sample of the chilled margin is likely to have preserved the composition of the magma making up the interior of the intrusion.

Zone showed that amphibole would crystallise at that level if water pressures were greater than about 1,500 bar. The absence of primary hydrous minerals in the intrusion indicates that this is an upper limit for the water pressure that could have prevailed during crystallisation.

Nature of the original Series

At the time the Layered Series crystallised, imperfect fractionation resulted in small amounts of liquid being trapped interstitially between cumulus crystals on the floor of the magma chamber. This trapped liquid now forms interstitial grains and overgrowths on the primary precipitates. Theoretically, it can be restored to its original liquid state if the rock is returned to the conditions of crystallisation of its cumulus phases. Rocks that are best suited for this purpose are those containing relatively high proportions of elements which are excluded from the cumulus minerals and provide a measure of the amount of trapped liquid⁸, such as Zr, Rb, Hf and K.

Fig 3 Comparison of the experimentally determined liquid compositions (solid curves) for SiO_2 and iron oxide at successive stages of crystallisation of the Layered Series with the values calculated by Wager¹ (broken curves).



In practice several factors could intervene during crystallisation and cooling to alter the composition of any liquid that would be restored artificially, so the glass produced by quenching partially melted Layered Series rocks will differ somewhat from the original trapped liquids. The compositions obtained by this method must be tested by preparing synthetic material corresponding to the composition of the artificially restored liquid and ascertaining whether the proper minerals appear on the liquidus at a uniform temperature appropriate for the stage of differentiation at which those same minerals crystallised in the intrusion. If they do not, the liquid composition or the conditions of temperature and oxygen fugacity must be incorrect. By varying one or more of these factors the natural and synthetic phase relationships can eventually be matched.

Such a procedure of tedious trial and error adjustments ultimately yielded consistent relationships between the natural rocks and liquids that could have produced them. The solution

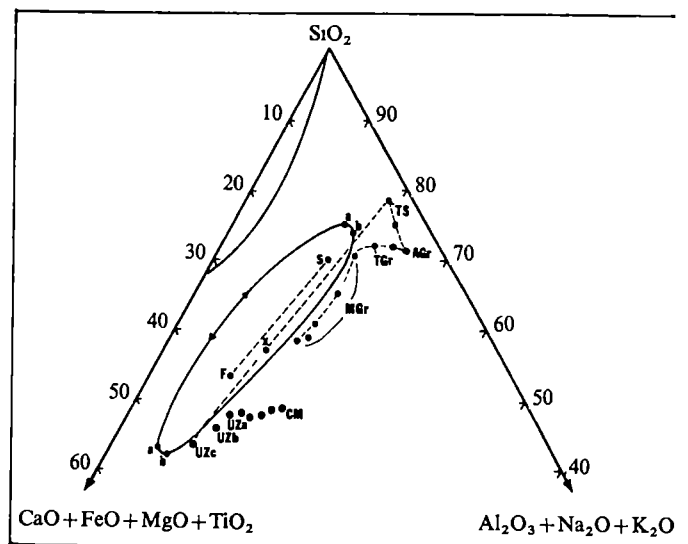


Fig 4 Compositional relations of Skaergaard liquids. Diagram is the same as that used by Weiblen and Roedder¹⁶ except that TiO_2 has been included with the mafic components $\text{MgO} + \text{Ca} + \text{FeO}$. Composition x was prepared by mixing and homogenising two parts by weight of the liquid of Upper Zone C with one part granophyre of the Tinden Sill. F and S are compositions of coexisting glasses in the same sample after it was held at a temperature about 5°C above its liquidus and quenched. a, a' and b, b' are pairs of coexisting immiscible liquids found by Roedder in the system Fayalite-Leucite-Silica. MGr, TGr and AGr are melanogranophyres, transitional granophyres and acid granophyres from the Layered Series, and TS is the granophyre of the Tinden Sill. Broken lines through the granophyric compositions indicate probable boundaries followed by liquids descending to a ternary minimum at AGr. The distinctive trend of the Tinden Sill is explained by progressive melting of gneiss with more silica than AGr.

obtained by this method is not unique, unfortunately, because there are several variables that cannot be evaluated, and if some condition has been misjudged or neglected the liquid composition obtained could be in error even though it gives consistent results. As our understanding of the conditions of crystallisation of the intrusion are improved, we can expect that better estimates for the liquid compositions will be possible.

Details of this work will be reported elsewhere, but several features of the experimentally defined liquids differ from those calculated by Wager and merit comment (Fig 3). Instead of being steadily enriched in silica, the late stage liquids are strongly depleted in this component. On the other hand, enrichment of iron was even stronger than Wager postulated. As Chayes⁴ deduced by other means, there was little if any enrichment of alkalis.

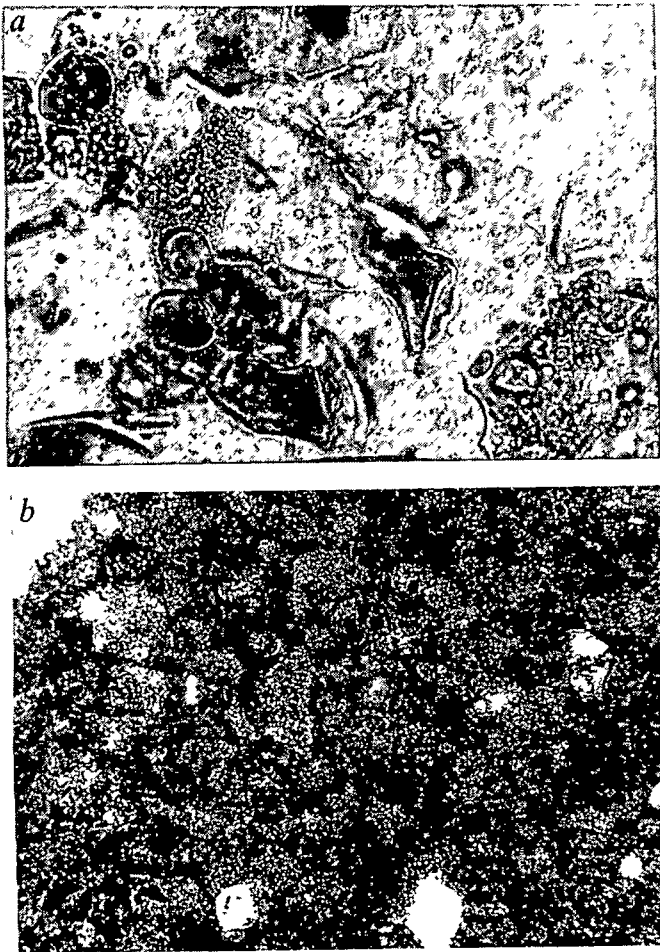


Fig 5 Typical textures in quenched glasses produced when compositions intermediate between liquids of the Upper Zone and granophyre were first homogenised at 1,375 °C then run at temperatures just above their liquidus *a*, Two parts granophyre and one part Upper Zone C, *b*, textures obtained under the same conditions from compositions in which the proportion of the granophytic component is small compared to that of the Upper Zone liquid. Width of fields is 0.12 mm

Of more importance, however, is the discovery that the trend of differentiation in the Upper Zone was in part governed by an immiscible relationship. Others have speculated on immiscibility in the Skaergaard intrusion^{9,10,19}. This possibility attracted attention during the course of experimental work at the University of Edinburgh in 1972 when it was found that synthetic compositions prepared from coprecipitated gels¹¹ with compositions close to those of the Upper Zone liquids could not be homogenised at temperatures slightly above their normal liquidus. The close similarity between these liquids and those found to be immiscible in lunar rocks formed under similar conditions¹², together with the fact that the liquid of Upper Zone C and a typical granophyre had almost the same liquidus temperatures (near 1,000 °C), reinforced this suspicion. As in the case of lunar rocks, one of the conjugate liquids is rich in iron and phosphorus and low in silica while the other is rich in silica and alkalis but low in iron.

This hypothesis was thought to be untenable at one stage¹³ when difficulty was encountered in duplicating the immiscible separation in liquids that had first been homogenised at higher temperatures. It is now known, however, that this was the result of the form of the liquidus and solvus boundaries and the fact that small differences of composition may cause the contrast between coexisting liquids to be drastically reduced or even eliminated. Another factor arguing against immiscibility was the evidence of trace element and isotopic data (G G Goles and E J Dasch, unpublished) that indicates

that the large granophytic bodies within the Upper Border Group could not have been derived, at least in large part, from the same parental liquids that formed the gabbros. Instead, they have stronger affinities to the basement gneisses.

After further study that narrowed the possible errors in liquid compositions, temperatures and oxidation conditions, it has become clear that the Upper Zone liquids followed the boundary of a two-liquid field close to that outlined by Roedder¹⁴ and illustrated in Fig 4. Liquids rich in iron that precipitated the mineral assemblages of the Upper Zone have an immiscible relationship to rocks rich in silica such as the melanogranophyres found in the same horizons and in the Upper Border Group. Compositions intermediate between these end members were prepared by repeated fusion, grinding and homogenisation at temperatures near 1,375 °C. They were then placed in unsealed silver-palladium envelopes and held for periods of 1–10 h at temperatures about 5–10 °C above their liquidus in an atmosphere controlled by mixtures of hydrogen and carbon dioxide. Oxygen fugacities were close to those estimated for corresponding levels by Williams⁶ and were calibrated against quartz-fayalite-magnetite and magnetite-wüstite reaction curves. Unmixing was observed in runs as short as 1 h, but the size of globules and compositional contrasts increased in longer runs.

Typical textures are illustrated in Fig 5. Two distinct glasses were readily produced from compositions close to the end member rich in silica. More iron-rich compositions produced finer textures consisting of clouds of small droplets of glass and possibly cristobalite, similar to those reported by Grieg¹⁵.

Compositional relations are shown in Fig 4. Early liquids corresponding to the Lower and Middle Zones cross the central part of the diagram as they become progressively depleted in feldspar components and enriched in iron. They reach the solvus and change their course to a trend away from silica, alkalis and alumina. The compositions of typical melanogranophyres are complementary to this trend and lie on the opposite side of the solvus. Acid granophyres seem to have evolved farther down the liquid line of descent between the solvus and an invariant point close to the $\text{SiO}_2\text{--Na}_2\text{O}+\text{K}_2\text{O}$ side of the diagram. The density contrast between the two immiscible liquids is marked (approximately 2.8 for the ferro-gabbro compared to less than 2.4 for the granophyres, according to unpublished measurements by T. Murase) and the viscosities of the iron-rich liquids must have been low (about 10^3 poise, Murase, unpublished), so that gravity separation of two such liquids could have been efficient. Small irregularly shaped granophytic bodies are common in the upper horizons of the Layered Series and in the lower units of the Upper Border Group (Fig 6).

Fig 6 Typical outcrop in the upper part of Upper Zone C showing irregular felsic bodies interpreted as partially segregated immiscible liquids.



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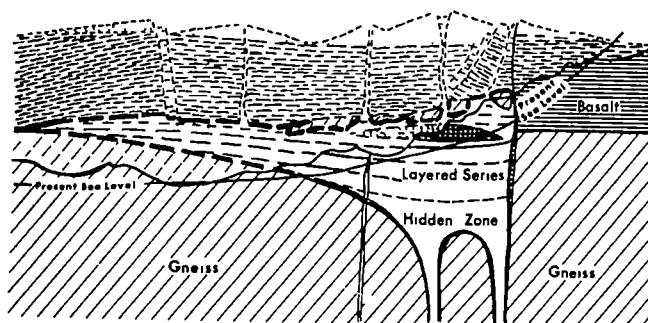


Fig 7 A schematic north-south section through the Skaergaard intrusion showing structural and volumetric relationships consistent with known data

As a further test of the experimentally determined liquid compositions, one can calculate the compositions and proportions of mineral assemblages that must be removed from each liquid to proceed to its successor. These fractionated assemblages should match corresponding units of the Layered Series and Border Groups for appropriate levels. When mass balance calculations of this kind are carried out for the new Skaergaard data, it is found that consistent solutions are obtained, but there is some uncertainty in the composition and relative volumes of the Border Groups, for which few data are available. For this reason, a reliable solution of the volume problem must await a thorough study of additional samples collected in 1974, using data obtained so far, it has been possible to make preliminary estimates of the relative volumes of the principal units of the intrusion. By relating these proportional volumes to measured stratigraphic thicknesses one can estimate the lateral extent of each layer and from this the form of the intrusion. Figure 7 illustrates a schematic section that is consistent with geophysical data and average compositions of the rocks and liquids as they are known at this time.

Apart from the great reduction of the volume of the Hidden Zone, the principal differences between these and previous estimates are in the greater lateral extent of the middle portions of the intrusion from Lower Zone B through the Middle Zone.

A further complication arises from the recent discovery that the phase layering of the Layered Series transgresses structural and stratigraphic horizons. The sequence of appearance of mafic phases is displaced upward in the eastern part of the intrusion relative to a section on the west side, so that cumulus clinopyroxene and magnetite appear and olivine disappears at levels that rise toward the east relative to distinctive horizons, such as the conspicuous Triple Group. The fact that the composition of plagioclase does not seem to be affected by this phenomenon suggests that the liquidus temperatures of iron-bearing minerals reflected a difference in oxygen fugacity from one side of the intrusion to the other. This feature was only recognised very recently and requires more study.

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Computer simulation of protein folding

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A new and very simple representation of protein conformations has been used together with energy minimisation and thermalisation to simulate protein folding. Under certain conditions, the method succeeds in 'renaturing' bovine pancreatic trypsin inhibitor from an open-chain conformation into a folded conformation close to that of the native molecule.

PROTEIN molecules owe their enormous functional versatility to the fact that they spontaneously fold into complicated and unique conformations determined by the particular amino-acid sequence¹. Discovering the relationship between protein sequence and conformation is a fascinating theoretical problem of fundamental importance. Most previous theoretical work has used the concept of 'local structure', in which the conformation of a short segment of polypeptide chain is supposed to depend almost entirely on the sequence of that segment. Although this approach has helped understand local secondary structure^{2,3}, it has not shown how residues distant along the chain can come together to form the overall conformation. The only promising attempt to study the tertiary folding of a

protein, in this case myoglobin, was based on the packing of cylinders supposed to represent α helices⁴. The method was not implemented on a computer and cannot be applied more generally to other proteins not built entirely from helices.

Here we tackle the problem differently. First, we simplify the representation of a protein by averaging over the fine details. This is done both to make the calculations much more efficient and also to avoid having to distinguish between many conformations that differ only in these finer details. Second, we simulate the folding of this simple structure by the combined use of convergent energy minimisation and normal mode thermalisation, which accelerate the process by avoiding the many non-productive random fluctuations that occur in nature. Tests of the procedure on bovine pancreatic trypsin inhibitor (PTI), show that under certain conditions it can rapidly reproduce the correct overall folding of this small protein molecule.

Simple representation of protein structure

Even the smallest protein (say 50 residues) is extremely complicated, with about 750 atoms and 200 degrees of freedom (single-bond torsion angles). Calculating its free energy presents severe computational difficulties, in particular when considering interactions with the rapidly moving solvent molecules and the

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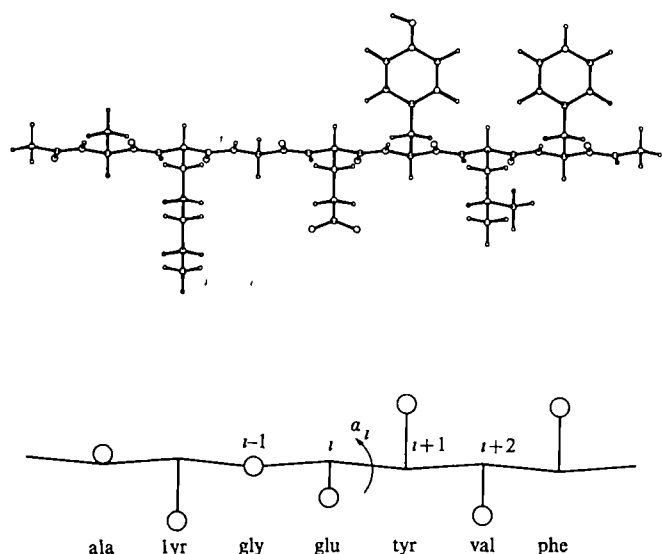


Fig. 1 Relationship between the simplified model of protein structure introduced here and the real all-atom structure of proteins. The two reference points for each residue in the simplified model correspond to the centroid of the side chain and the C^α . Each residue is only allowed one degree of freedom, the torsion angle α between the 4 successive C^α s of residues ($i-1, i, i+1, i+2$). All the side chains of a given type have the same simplified geometry. The bond lengths, bond angles, and torsion angles used to define the geometry of the simplified molecule were taken as the average values found in eight protein conformations, though they could just as well have been taken from amino-acid model compounds.

thermal motion of parts of the protein itself. Our method is designed to overcome these problems and is based on two assumptions: (1) that much of the protein's fine structure can be eliminated by averaging, and (2) that the overall chain folding can be obtained by considering only the most effective variables (those that vary most slowly yet cause the greatest changes in conformation).

Averaging over groups of atoms in the full structure gives a simplified structure with each residue represented by only two centres, the C^α atom, and the centroid of the side chain. Interactions are assumed to occur only between side chains, while the C^α positions define the chain path (Fig. 1). Each amino-acid residue only has one degree of freedom, the torsion angle about the line joining two adjacent C^α s (known here as α). Although a simple representation based on virtual bonds has been used before to study polypeptide random coils⁵, it has never been applied to ordered globular proteins. This simplification reduces the degrees of freedom by a factor of four and the number of interaction centres by a factor of fifteen. One might also hope that the reduced space used here to describe different conformations would have many fewer energy minima. The space is of lower dimension and the side chains are smooth spheres without all the minor bumps of the all-atom structures.

The effect of the fine details and more rapidly changing variables is included in the effective time-averaged potential functions used. (By the ergodic theorem⁶, this time averaging is equivalent to Boltzmann weighted spatial averaging over conformations generated by changing the fast variables.) For rotations about the torsion angle α , the effective potential is obtained by averaging the energy over all those conformations of a dipeptide that have a particular value of α . As it was impossible to study all 400 different dipeptides, calculations were done on six considered most representative ala-ala, ala-gly, ala-pro, gly-gly, gly-ala and pro-ala. This showed that the effective potential only depended on the nature of the second amino acid, giving different potentials for the α preceding ala, gly, and pro. The alanine potential had a deep minimum at $\alpha = 210^\circ$ (twisted β chain) and a more shallow minimum at $\alpha = 45^\circ$ (α helix), the glycine potential had a

broad minimum at $\alpha = 0^\circ$ (reverse turn), and the proline potential had two sharp minima at $\alpha = 60^\circ$ and $\alpha = 210^\circ$. Because aspartic acid and asparagine were found to occur as frequently in the reverse turns of known protein conformations as glycine, the same potential was used for all three. The alanine-type potential was used for all other amino acids except proline. All the atoms were included in these dipeptide calculations, which used energy parameters derived from crystals of amino acids, amides, and hydrocarbons.

The interaction potential between a pair of identical amino-acid side chains was also calculated by spatial averaging. Each side chain was assumed to be spherically symmetrical with a radius equal to the average radius of gyration of that side chain. The effective potential was calculated at various distances apart as a sum of the interaction energy of all atoms anywhere in one sphere with all atoms anywhere in the other.

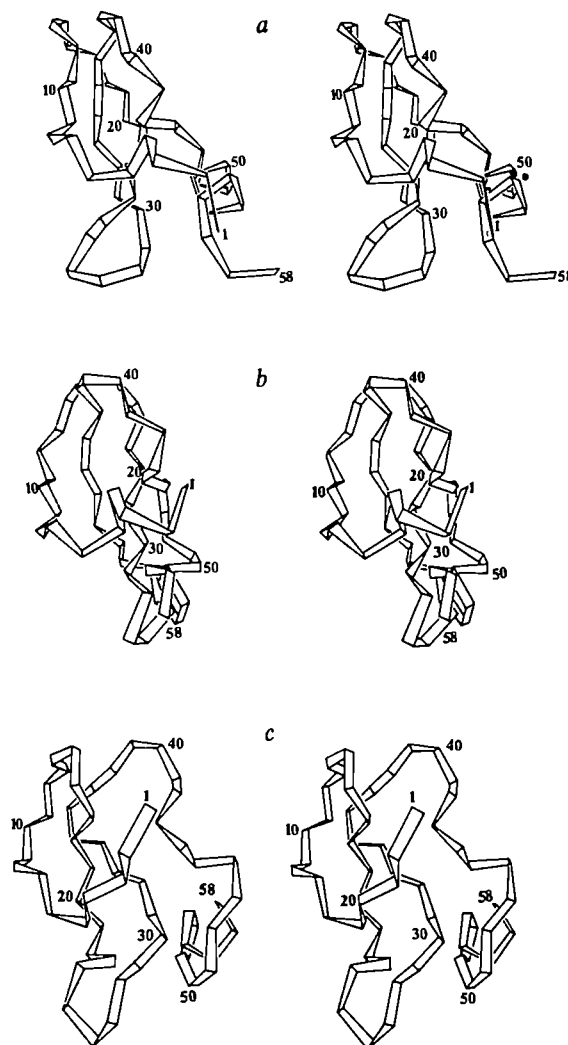


Fig. 2 Stereo ribbon drawings of PTI in *a*, the idealised native molecule, *b*, the minimum energy conformation generated starting at the idealised native conformation and *c*, the best conformation generated by folding from an extended chain with a terminal helix (the final conformation in Fig. 4). (The programs used to rotate the molecules into the same orientation and then draw the ribbon between C^α s were provided by Dr A. D. McLachlan.)

sphere This effective potential between identical side chains was approximated by a Lennard-Jones type function, and potentials between pairs of different side chains were obtained by a geometric mean combining law. Because proteins fold in water not a vacuum, interactions with the solvent are included by assigning to each side chain a hydrophobic energy taken from the solubilities of amino acids in water and in ethanol.⁷ In the calculations, the energy of transfer between these two solvents was taken as the difference in energy of the side chain when isolated in water and when completely surrounded by other residues. When surrounded by an intermediate number of neighbours, the hydrophobic energy was varied according to a sigmoid function. More complicated models that include hydrogen bonds and S-S bridges will be described elsewhere, together with full details of the standard geometry and all energy parameters used.

The folding of this idealised protein can be simulated by solving the equations of molecular dynamics at sufficiently small time intervals. In a viscous medium like water, these equations of motion can be approximated by Langevin equations, where the change in the variables is directed down the energy gradient with a random deflection due to Brownian motion.⁸ For greater computational efficiency we neglect these thermal fluctuations while the chain folds, and the end point of the trajectory is the potential energy minimum accessible from the starting conformation. We minimise the energy of the idealised protein chain with respect to all the α angles using a powerful quadratically convergent method (VA09A, by R. Fletcher and taken from the Harwell Subroutine Library). After reaching a minimum, thermal fluctuations are reintroduced and the conformation is considered to be vibrating about the minimum so that each normal mode has average kinetic energy $kT/2$ (where k is the Boltzmann constant and T the absolute temperature). A new starting conformation for the next pass of energy minimisation is chosen by suddenly stopping the thermal vibration. At this time each normal-mode coordinate will be displaced randomly from the minimum by $(R(n)kT/\lambda)^{1/2}$, so that the associated energy becomes $R(n)kT/2$. Here λ is the eigenvalue of the energy second derivative matrix corresponding to the particular normal mode, and $R(n)$ is a random number uniformly distributed between 0 and 1. (An exponential distribution of random numbers between 0 and ∞ would be more realistic.) Normal-mode thermalisation avoids non-productive changes in conformation for it knows which combinations of angle changes should cause the greatest change in conformation for a given energy increase.

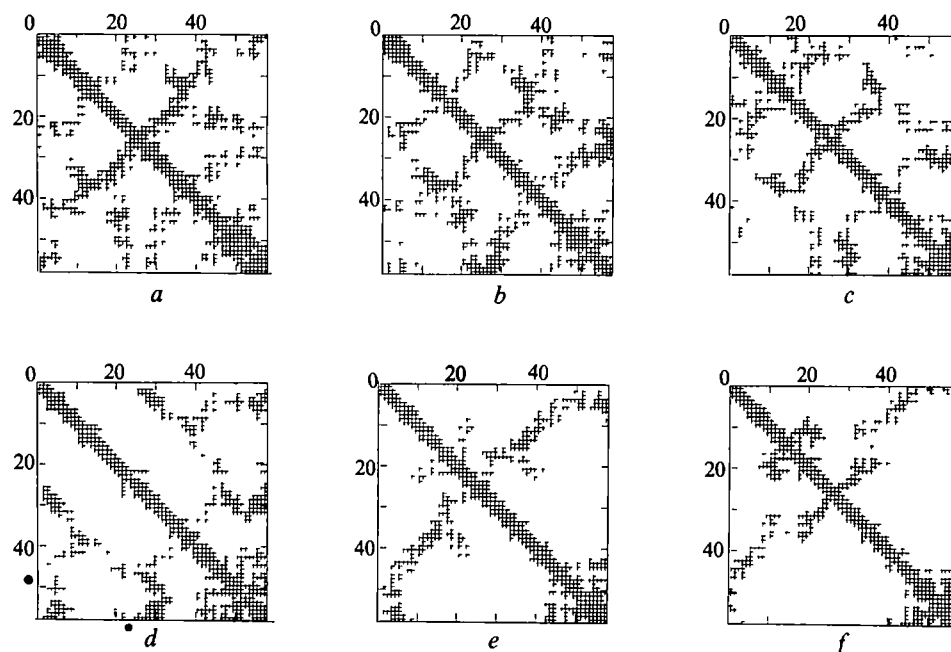


Fig 3 Contact maps¹¹ of the following conformations *a*, idealised native, *b*, folded from idealised native, *c*, folded from extended chain with terminal helix, *d*, *e* and *f*, folded from the same starting angles and with the same parameters as *c*, but using different sequences of random numbers for the thermalisation. The six structures shown here have energies of -332.0, -52.0, -48.9, -44.9, -32.5, -28.7 kcalorie mol⁻¹, respectively and r.m.s. deviations from the idealised native conformation of 1.1, 3.4, 5.3, 6.3, 11.7 and 12.4 Å respectively. (The energy of the idealised native conformation is so high because it has not been minimised.) A cross at the intersection of row i and column j indicates that residue i is within 10 Å of residue j . In these maps, helices feature as a broadening of the diagonal (down from top left to bottom right), antiparallel β sheet as a band perpendicular to the diagonal, and parallel β sheet as a band running parallel to the diagonal.

Testing the simplified representation

The drastic simplifications used in the present representation of a protein conformation were tested by minimisation from near the native folded conformation. Bovine pancreatic trypsin inhibitor was chosen for this test as it is the only small protein (less than 100 residues) of known conformation that has a single polypeptide chain and no additional prosthetic group. As a first step, a simplified native PTI conformation was obtained by taking the C α positions and side-chain centroids from the X-ray coordinates⁹ (kindly supplied by Drs Huber and Steigemann). Next an idealised chain, based on the PTI sequence and having the same geometry for all side chains of the same type, was made to fit the simplified native coordinates by adjusting the α torsion angles. This conformation, known as the idealised native structure, deviates by 1.1 Å r.m.s. from the simplified native structure. The r.m.s. deviation is

$$\left\{ \frac{1}{N} \sum_{i,j} (\Delta r_{ij})^2 \right\}^{1/2}$$

where Δr_{ij} is the difference, in the two structures, of the distance between side-chain centroids (i and j). Energy minimisation from this starting conformation was then carried out to reproduce the stability of native PTI.

After 558 cycles a perfect minimum is reached at an energy of -52.0 kcalorie mol⁻¹ and a r.m.s. deviation of only 3.37 Å from the simplified native conformation. Thermal randomisation about this minimum does not lead to further movement from the native molecule on subsequent minimisation. Randomly disturbing the initial best-fit angles (with a disturbance between -15° and +15°) has little effect on the conformation obtained by subsequent minimisation. Figure 2 compares the minimum energy and native chain folding in stereo, and Figure 3 compares the contact maps. Because of a general twisting of the molecule, the comparison of the two structures should be done in stereo. Because main-chain hydrogen bonds have been omitted, the terminal helix becomes distorted and consequently packs too tightly against the β hairpin centred at residue 27.

Simulation of PTI folding

Having shown that so simple a model can represent the stable conformation of a folded protein, we tried to simulate the actual process of folding. Most tests were done with two open

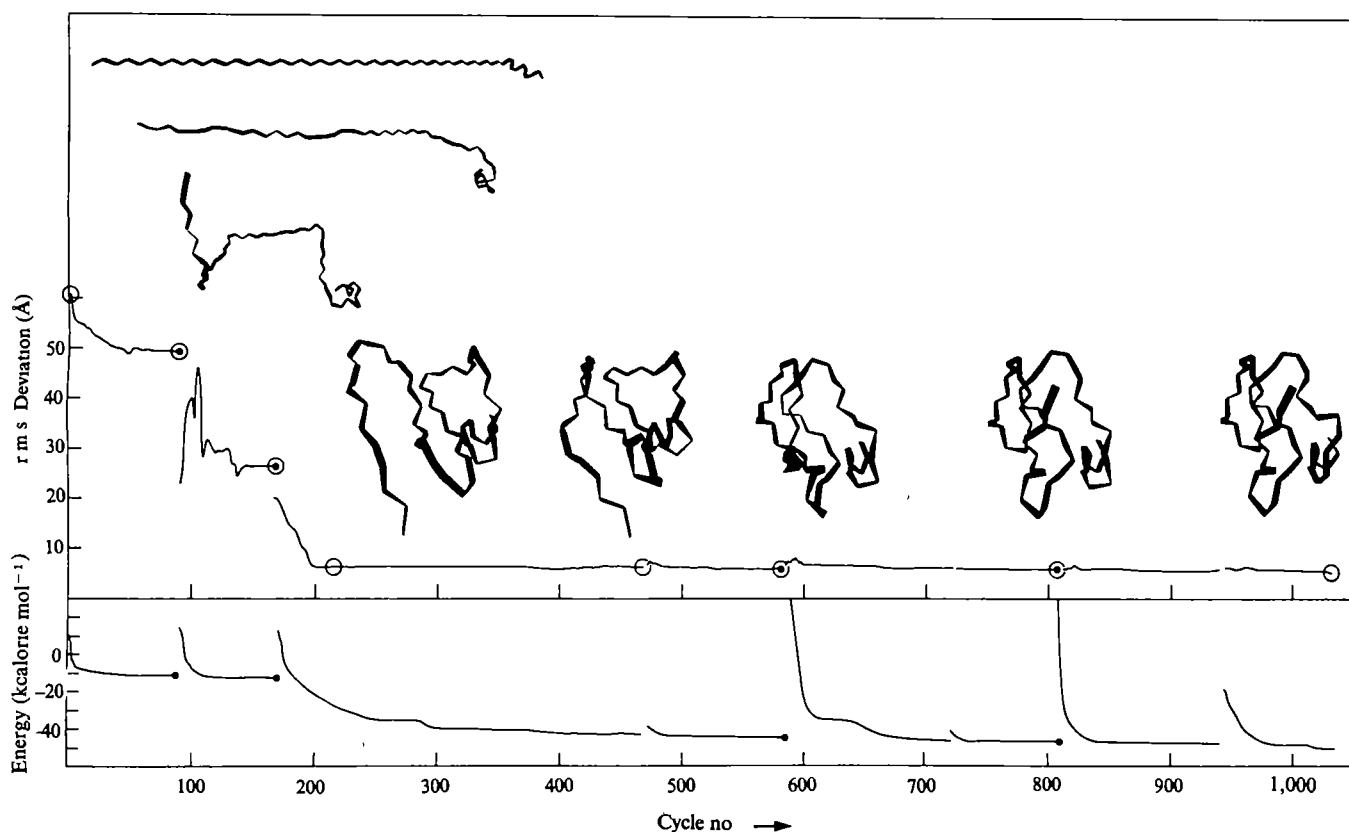


Fig. 4 Simulation of PTI folding from an extended starting conformation with the terminal helix ($\alpha = 180^\circ$ for all except 48 to 58 where $\alpha = 45^\circ$). No knowledge whatsoever about native PTI is used during this simulation (apart from setting the terminal helix). The conformation was thermalised at the end of each minimisation except near cycles 490 and 730 when the energy rises slightly because the minimisation was restarted after rounding the torsion angles to one degree. In the first two thermalisations, each normal mode was perturbed in the plus direction to raise the associated energy to $R(n)kT/2$ with $T = 1,000$ K. In the other three thermalisations, the perturbations were randomly in the plus and minus directions but always such as to raise the energy by $kT/2$ with $T = 300$ K. (Because the random numbers are distributed uniformly rather than exponentially, these temperatures do not correspond to the macroscopic temperature.) The 8 ribbon diagrams, which show the C^α chain path, refer from left to right to the 8 conformations at the circled points on the r m s deviation curve, respectively. The last five conformations have progressively lower energies and are each a little closer to the native structure ($E = -43.3, -45.7, -46.0, -46.9$ and -48.9 kcalorie mol^{-1} , respectively, r m s deviation = 6.08, 5.7, 5.6, 5.4 and 5.3 Å, respectively). The solid dots at the end of a minimisation indicate that a perfect minimum was reached (r m s gradient less than 10^{-6} kcalorie $\text{mol}^{-1}\text{rad}^{-1}$). One cycle takes about 0.6 s on an IBM 370/165 computer.

starting conformations, one fully extended (all $\alpha = 180^\circ$), and one extended apart from the C-terminal helix ($\alpha = 180^\circ$, except for residues 48 to 58 where $\alpha = 45^\circ$). Retaining the terminal helix from the native structure is justified here as we are more concerned with the process of folding than with prediction of the native conformation of an unknown protein. In the latter case a statistical rule (see ref. 2) could be used to guess the position of the α helices in the starting conformation. Figure 4 shows the iteration history of minimisation from the second of these starting points, which was the most successful run of those obtained to date. Thermal randomisation about the first minimum, an irregular but extended conformation, raises the energy and in this case causes the chain to bend back on to itself decreasing the r m s deviation. From this point, minimisation first opens the molecule again, but then reaches a new minimum where the chain now has kinks that could become the bends of β hairpins. After a second randomisation, minimisation rapidly folds the molecule bringing the terminal helix close to the β hairpin centred on residue 27. More minimisation and thermalisation first brings together the two top loops (near residues 15 and 40), and then brings the N-terminal tail on to the rest of the molecule. The final folded conformation of Fig. 4 is remarkably like the native molecule (Figs 2 and 3). In both conformations the chain bends back on itself near residues 14, 27, and 40. In both conformations, the pairs of half-cystine residues that are experimentally known to form S-S bridges, are close together (<10 Å). It is interesting that the C-terminal helix is the part

of the native molecule reproduced least well even though these residues had been set to a perfect helix in the starting conformation, this is due to the omission of peptide-peptide hydrogen bonds which stabilise the helix and could now be introduced.

Repeating the folding simulation from the fully extended starting conformation also lead to a compact structure with many of the features of native PTI, although after the same number of cycles the r m s fit was a little worse (7.7 Å instead of 6.5 Å) and the energy was higher (-27.7 kcalorie mol^{-1} instead of -44.9 kcalorie mol^{-1}). Almost all the differences in conformation of these two folded structures involved the last 10 residues which remained extended if not pre-set to a helix and consequently failed to pack against the rest of the molecule.

Variation of folding conditions

Changing either set of starting torsion angles by a random value between -15° and 15° had little effect on the final conformation. Folding at a lower initial temperature ($T = 300$ K, rather than $T = 1,000$ K) failed to reach a compact conformation, as the thermal disturbances were too small to get out of the local minima corresponding to an extended chain.

Four additional runs of 600 cycles, under the same conditions as those used in Fig. 4 but based on different sequences of random numbers, gave rise to different folded shapes. In one of these, the folded molecule was close to the native structure (r m s deviation of 6.3 Å), although the β sheet was formed between parallel rather than antiparallel chains (Fig. 3f). In

Two others, the antiparallel β sheet centred near residue 27 was formed, but this hairpin did not subsequently fold on to itself to give a compact shape (Fig 3d and e). Conformations that deviated more from the native structure always had higher energies, which gives an independent criterion for choosing the best conformation and suggests that more passes of thermalisation and minimisation lead to a conformation closer to the native one. Of the five runs using different random numbers for the thermalisation step, two succeeded in getting to within 6.5 Å of the simplified native structure in less than 600 cycles. That certain folding pathways are less successful is consistent with the experimental results of Creighton¹⁰ who has analysed the predominant kinetic intermediates present at different times after starting PTI renaturation and found several with the wrong tertiary fold.

General model for protein folding?

It seems remarkable that so simple a model based on time-averaged forces can account for the stability and folding of a molecule as complicated as a protein. Looking at known protein conformations closely, one is struck by the precise geometry of the interatomic contacts that stabilise the molecule: all possible interior hydrogen bonds are well formed, and many of the nonpolar side chains interlock to form a close-packed interior. As the forces responsible for this precise geometry fall off rapidly with distance and improper orientation, it would seem that folding must depend on a very rare random fluctuation that happened to bring the right residues close together with sufficient precision for the short-range forces to take effect. It therefore seems unlikely that these short-range forces could 'direct' the folding from an open disordered structure. In view of the present results, however, the time average of these short-range forces may play an important role in directing protein folding. These effective forces, which are weak, fairly long range, and not too dependent on orientation, restrict the number of low energy conformations severely, they cause the chain to fold into the approximate shape rapidly and without having to pass through many local minima. Because this approximately folded molecule corresponds to a large region in the space of possible protein conformations, folding would not be so rare an event.

As a general model for protein folding we propose that initially, when the chain has a flexible open structure, the effective time-averaged forces between the residues play a

central role, folding the chain into a compact shape with most groups close to their final positions (say within 5 Å). Once the chain becomes compact with less freedom of movement, the specific short-range interatomic forces become important, they form a precise conformation provided that the resulting gain in enthalpy overcomes the loss in entropy. The process would be rather like crystallisation, with the atoms simply falling into place from their nearby positions in the approximate folded conformation.

To simulate this second step one switches over to progressively more detailed models gradually incorporating more atoms and ending with the all-atom structures considered in earlier work^{12,13}. Although calculating the energy of the all-atom molecule would be time consuming, one would have the great advantage of starting close to the right conformation and could minimise successive overlapping zones of a few residues at a time without having to search through many local minima.

The general concept of using a simple model based on effective time-averaged forces when the detailed forces are too complicated has many potential applications, for example, the formation of protein quaternary structure and multi-enzyme complexes, virus assembly and so on. At each level of complexity, forces would be time averaged over those substructures that are relatively fixed or seem to play a less important role in the assembly. Such a hierarchical approach might eventually lead to an understanding and simulation of very complicated biological assembly processes.

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letters to nature

Two kinds of stellar collapse

HERE I shall show that the events which produce compact objects may be divided into at least two classes on the basis of the impulse given to a binary system containing the collapsing object. This impulse may be related to the amount and symmetry of mass loss resulting from the collapse event.

If one member of a binary system in circular orbit loses mass in a spherically symmetric manner and on a time scale much less than the orbital period, then the barycentre of the remaining system acquires the velocity¹

$$S_g = \frac{M_1 M_2 (1-q)}{(M_1 + M_2)(qM_1 + M_2)} S_0 \quad (1)$$

where S_0 is the initial relative orbital velocity of the two masses,

M_1 and M_2 are the initial masses and qM_1 and M_2 are the final masses. If more than half of the total initial mass of the binary is lost the system becomes unbound, if not, the orbit becomes eccentric¹. If the initial orbit is eccentric, the explosion is asymmetric, or the effects^{2,3} of the collision of the debris with the companion star are included, then several more parameters are needed to describe the event and to determine the new orbit. Conservation of momentum arguments indicate that explosions producing loss of a few tenths of the binary mass, or giving the compact remnant a recoil velocity of several tenths of S_0 will generally (except for fortuitous combinations of parameters) lead to orbits of substantial eccentricity. If $M_1 \sim M_2$ then barycentre recoil velocities of a few tenths of S_0 will also result. Slightly more violent events produce hyperbolic orbits.

I first summarise the evidence for the expulsion of large

amounts of mass or momentum in the formation of some compact objects

(1) The newly discovered⁴ binary pulsar is in an orbit both small ($a \sin i = 6.9 \times 10^{10}$ cm) and eccentric ($e = 0.61$). Since close binaries usually⁵ have circular orbits it is likely that the eccentricity of the orbit is the result of the recent event which produced the neutron star. An event capable of inducing this large eccentricity in a circular orbit must involve the expulsion of large amounts of mass or momentum, and will also give the barycentre a velocity of a few tenths of S_0 if the two stars are of comparable mass, since $K_1 = 198 \text{ km s}^{-1}$. I can estimate $S_g \sim 100 \text{ km s}^{-1}$.

(2) The distance from the galactic plane^{6,7} of the Her X-1-HZ Her binary system requires that if it originated in the plane it acquired a component of S_g perpendicular to the plane of at least 100 km s^{-1} . Given the observed Her X-1 mass function, a present day Population II main sequence turnoff of $0.80 M_\odot$, the assumption that to initiate neutron star formation requires $1.40 M_\odot$ of degenerate material, a Population II main sequence mass-luminosity relation and a minimum credible neutron star mass of $0.10 M_\odot$, then a simple argument⁸ shows that the age of the system is much less than the age of Population II objects, and a halo (high velocity) origin is ruled out. It is reasonable to attribute the large value of S_g to the event which formed the neutron star, although more complicated scenarios are possible.

(3) The proper motion⁹ of PSR1133+16 implies a transverse velocity $\sim 380 \text{ km s}^{-1}$. There is also evidence⁹ for high velocities in several other pulsars. This indicates either tight binding in a now disrupted binary, or an asymmetric explosion of a single star, as the origin of the high space velocities of some (or all) pulsars.

There is also evidence for a second class of compact objects, formed in a much less disruptive manner. These are the two X-ray sources 3U1746-37 and 3U1820-30, which are respectively identified¹⁰ with the globular clusters NGC6441 and NGC6624. The X-ray error boxes are 0.0184 and 0.0060 square degrees, respectively. The X-ray and optical¹¹ positions agree to about $1'$ less than the size of the globular clusters. The probability of accidental globular cluster identification of X-ray sources with such small error boxes is difficult to calculate exactly because the X-ray error boxes and cluster sizes are distributed over a wide range. A rough argument indicates that the probability is less than 10^{-4} .

The escape velocities of these globular clusters are not known. Root mean square escape velocities have been determined^{12,13} for three clusters by measuring directly the dispersion of the velocities of individual cluster stars. The results are 16 km s^{-1} for 47 Tuc, 18 km s^{-1} for M92, and 20 km s^{-1} for ω Cen, but these must be regarded as only accurate to within a factor of 2, or perhaps taken as upper limits. Mass estimates¹¹ based on an assumed luminosity function lead to $r \text{ m s}^{-1}$ escape velocities around 10 km s^{-1} for a representative cluster.

An X-ray source produced in a globular cluster with a barycentre velocity of 100 km s^{-1} will leave the cluster in less than 10^5 yr . The source 3U1820-30 is observed¹⁰ to vary, which supports its identification as an accretional binary source, rather than as a supernova remnant. Since the lifetime of an old population binary X-ray source is at least the Kelvin time of stars of moderate mass (Her X-1, for example, required 10^7 yr to rise to its present height above the galactic plane), the presence of a source in a globular cluster requires that it be bound to the cluster. So the events which produced the globular cluster sources were very different in character from the events producing Her X-1 and the high velocity pulsars.

No conclusion may be drawn from the presence of the massive binary X-ray sources close to the plane, because a value of S_g of only about $30\text{--}50 \text{ km s}^{-1}$ is expected from a violent explosion^{3,14}. In the expected lifetime of these systems as X-ray sources they only travel $200\text{--}350 \text{ pc}$.

I denote events which produce high velocity compact objects as class A, and those producing low velocity compact objects as class B. It cannot yet be determined whether there is a continuum between these extremes. A variety of selection effects involving the observability of compact objects makes it difficult to discuss the statistics and formation rates of objects of the two classes. Most of the objects of class A are radio pulsars, and detectable only for that reason. One (Her X-1) is clearly a magnetic neutron star, but would be detected as an accretional X-ray source even were it non-magnetic, or were it a black hole. The two known objects of class B have not been reported to show regular X-ray periodicity of the Her X-1 variety. This suggests the speculation that objects of class A are neutron stars, but that those of class B are black holes into which an entire star has collapsed, without an outward-moving shock and without expulsion of matter. But calculations¹⁵ indicate that for some equations of state it is also possible to form a neutron star without expelling a supernova shell, present understanding does not justify further speculation.

The observation that two X-ray sources are in globular clusters (out of about 100 galactic sources) is remarkable even aside from the dynamical implications of their remaining bound to the cluster. The total mass of globular clusters in our Galaxy has been estimated¹¹ as $10^7 M_\odot$. This should be compared with masses¹⁶ of $2 \times 10^{10} M_\odot$ for halo Population II, and of $8 \times 10^{10} M_\odot$ for the intermediate and old disk Population II. This implies that the globular clusters have an X-ray luminosity to mass ratio about two orders of magnitude greater than that of the Galaxy as a whole, or of the central bulge which contains most of the X-ray sources.

It is clearly desirable to study the two identified X-ray sources and globular clusters further. In the 3U Catalog¹⁰ there are two more suggested globular cluster identifications: 3U1736+43 with M92 and 3U2131+11 with M15. The X-ray error boxes are large, so I have ignored these, better X-ray positions are needed.

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Origin of the optical emission from Sco X-1

Two groups have reported analyses of photometric^{1,2} and spectroscopic³ data of Sco X-1, which suggest a binary nature. Prior to these studies no persistent periodic variations of the X-ray flux have been found, but the first group¹ claims a periodic variation in the light curve of 3.93 d with a mean amplitude of 0.8 mag, and the second² claims variations of 0.22 mag in amplitude, with a period of 0.787 d.

These results are conflicting, which makes one cautious about accepting the reality of these periodic variations in the

light curve But one can nevertheless speculate about their nature

With the usual assumption that the visible light is emitted by a hot, optically thick plasma, which is also responsible for the X-ray emission⁴⁻⁶, it is difficult to understand these observations. The separation between the optical and X-ray emitting regions is also suggested by the simultaneous observations in these wavelengths⁷. Large changes in optical luminosity can occur while the X-ray flux remains nearly constant, although the correlations between times of X-ray and optical activity, which are not always observed, imply some connection between the two emitting regions⁷. Another striking feature is the delay of the X-ray flares with respect to the optical ones. Here I propose a model that could explain some of the observations.

I assume that we have a binary system very similar to the Her X-1-HZ Her system, that is, a degenerate star (neutron star), which is the X-ray source, and a non-degenerate star almost filling its Roche lobe. In this case, the heating of atmosphere of the non-degenerate star by the X-rays will produce a hot spot, which I assume to be responsible for most of the visible emission. I further assume, as a first approximation, that the X-ray emission is nearly isotropic.

In this case, the fraction f_x of the total X-ray luminosity absorbed by the primary star is

$$f_x = \frac{1}{2}[1 - \sqrt{1 - (R^*/D)^2}] \quad (1)$$

where R^* is the 'average' radius of the primary star and D is the separation between the components.

Similarly, the ratio y between the area of the hot spot and the total surface area is

$$y = \frac{1}{2}(1 - R^*/D) \quad (2)$$

In both formulae it is assumed that the deformation of the primary star is small.

The optical flux at maximum light, which I assume to be caused by the spot, is given by

$$\phi_V = (L_V/4\pi)(s/y)\exp(-\tau_V)/d^2 \quad (3)$$

where L_V is the spot luminosity in the V band, s is the fraction of the spot visible at maximum light (s depends on the geometry of the orbit, mainly the orbital inclination angle) and d is the distance.

Since at maximum light the magnitude of Sco X-1 is $V = 12.2$ mag, which corresponds to a flux $\phi_V = 4.2 \times 10^{-11}$ erg cm⁻² s⁻¹ (ref 8), one obtains from equation (3)

$$L_V = 2.4 \times 10^{34}(y/s)d_{\text{kpc}}^2 \text{ erg s}^{-1} \quad (4)$$

To obtain this number I have assumed also that the interstellar absorption in the direction of Sco X-1 is $A_V = 1.5$ mag. This is consistent with counts of galaxies⁹ and also with the width of the Ca II line¹⁰.

On the other hand, if the spot radiates like a blackbody, one can write

$$L_V = 4\pi R^2 y \pi B_\lambda(T_e) \Delta\lambda_V \quad (5)$$

where T_e is the effective spot temperature and $\Delta\lambda_V$ is the effective width of the V filter.

Correcting the observed colours of Sco X-1 for the assumed reddening, I obtain the following range of variation

$$\begin{aligned} (U-B)_0 &\simeq -1.28 \text{ to } -1.08 \\ (B-V)_0 &\simeq -0.32 \text{ to } -0.28 \end{aligned}$$

These values are compatible with a spectral type varying from O5 at maximum light to B0 at minimum light.

Assuming that the effective temperature of the spot is about 37,000 K, corresponding closely to the spectral type at maximum

light, from equation (5) I obtain

$$L_V \simeq 3.9 \times 10^{34}(R^*/R_\odot)^2 y \text{ erg s}^{-1} \quad (6)$$

Now, combining equations (4) and (6)

$$(R^*/R_\odot)^2 s \simeq 0.6 d_{\text{kpc}}^2 \quad (7)$$

In a similar fashion we can work with the bolometric luminosity, giving

$$4\pi R^2 y \sigma_B T_e^4 = 4\pi f_x d^2 \phi_x \quad (8)$$

where σ_B is the Stefan-Boltzmann constant and ϕ_x is the observed X-ray flux ($\phi_x \simeq 3 \times 10^{-7}$ erg cm⁻² s⁻¹ in the 1-10 keV range). Numerically, equation (8) gives

$$(R^*/R_\odot)^2 y \simeq 5.2 d_{\text{kpc}}^2 f_x \quad (9)$$

From equations (7) and (9)

$$f_x \simeq 0.117 y/s \quad (10)$$

I assume as a reasonable value $s \simeq 0.6$. Therefore, using equations (1) and (2), in order that equation (10) be satisfied,

$$R^*/D \simeq 0.45 \quad (11)$$

If the non-degenerate star nearly fills its Roche lobe, the numerical value given by equation (11) implies a mass ratio $M_s/M_x \sim 2.2$, where M_s is the primary star mass and M_x is the mass of the X-ray source.

In this case, if the X-ray source is a neutron star with a typical mass of $1M_\odot$, the total mass of the system is about $3M_\odot$.

From Kepler's third law

$$D/R_\odot \simeq 4.2[(M_s + M_x)/M_\odot]^{1/3} P^{2/3} \quad (12)$$

where P is the orbital period in days.

Accepting that $P \simeq 0.78$ d (ref 2), equations (12) and (11) yield, respectively $D \sim 5.1R_\odot$ and $R^* \sim 2.3R_\odot$.

In this case, from equation (7) one obtains a distance of about 2.3 kpc for Sco X-1 and a luminosity in the X-ray region of about 1.7×10^{38} erg s⁻¹. This value indicates that the luminosity has reached the Eddington limit and that radiative forces play an important role in the accretion flow.

On the other hand, if we accept the results reported by Martynov¹ ($P \simeq 3.93$ d) equations (12) and (11) give values for the separation between the stars and for the primary radius which are a factor of three greater than those obtained with the data of Gottlieb *et al*. These values imply a distance greater by a factor of three and an X-ray luminosity greater by an order of magnitude. In this case, there is a difficulty since the calculated luminosity is greater than the Eddington limit.

Therefore, this model implies that orbital periods of less than 1 d are required. Gribbin¹¹ has suggested that Sco X-1 could be similar to the WZ Sge system, predicting an orbital period of about 4 h.

The bolometric spot luminosity is about 5.3% of the X-ray luminosity, $\sim 9.4 \times 10^{36}$ erg s⁻¹. Since the effective temperature is high, the ultraviolet radiation from the spot is strong enough to ionise the interstellar medium to the extent that the emission measure is 33 cm⁻⁶ pc (ref 12) if the interstellar gas density is about 0.8 cm⁻³ in the vicinity of Sco X-1. This emission measure compares well with the value obtained by Johnson¹³ from H β measurements around Sco X-1 and solves the problem of its theoretical explanation. Silk *et al*¹⁴ were not able to explain it by assuming that the ionisation is caused by X rays.

On the other hand, the model faces other difficulties such as the observed delay between optical and X-ray flares. These observations eliminate the possibility that the optical flares

arise from the relaxation of the atmosphere after irradiation by X-ray flares, as the model would predict. One might also suppose that the optical flares would be produced near the Lagrangian point, by instabilities in the mass flow induced by the X rays. These instabilities will later reach the X-ray emitting region, producing X-ray flares. But the observed time delay requires a propagation velocity of the instabilities of about 10^4 km s^{-1} , which is too high.

Although these and some other observed features cannot be explained, the model has some merits in predicting the masses and dimensions of the system as well as its distance. The calculated parameters are very similar to those of the Her X-1-HZ Her system, possibly indicating the existence of a class of binary X-ray sources associated with Population II objects.

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plane of the subsatellite orbit is determinable and is less than $1.3 \times 10^{18} \text{ gauss cm}^3$ it is unlikely that the component perpendicular to it is appreciably greater. Russell *et al*² estimated the error to be $1.33 \times 10^{18} \text{ gauss cm}^3$, and therefore concluded that the lunar magnetic moment is not significantly different from zero. Paradoxical as it may seem, it follows from this observation that the Moon possessed a magnetic field of internal origin in its early history.

Stable remanent magnetisation discovered in lava samples collected during the Apollo 11 mission has been interpreted³ as thermoremanent magnetisation acquired when the lava flows cooled through the Curie point 3.6 Gyr ago. It was concluded³ that a general lunar magnetic field of $\geq 1,000 \gamma$ was responsible. As I had proposed in a theory of the non-hydrostatic lunar figure⁴ that the Moon possessed a small iron core of 150–500 km radius, it seemed reasonable to suggest that a dynamo process within this core accounted for the field (Fig 1a). The disappearance of this magnetising field can be explained⁵ either the magnetic Reynolds number became subcritical or the core solidified during the last 3.2 Gyr.

To assume an iron core implies that the accreted Moon was heated to its melting point soon after its origin. It is not clear, however, that there was a sufficient heat source but it is possible⁶ that the Moon had formed cold and was not heated at depth in its early history so that the disseminated iron did not sink to form a core. Thus, the Moon, forming within a gas sphere, could have acquired a uniform permanent magnetisation (Fig 1b) from a primaeval field in the condensing solar nebula—a remnant of the galactic magnetic field lines which had not diffused away as the contracting dust cloud compressed the field. Such a general magnetic field within the Solar System would have vanished completely from interplanetary space when the gas was expelled from around the terrestrial planets during the T-tauri phase of the Sun. The postulated permanent magnetisation of the deep interior of the Moon would have produced a dipole field at the lunar surface which magnetised both the anorthositic highlands as they cooled after differentiation and the basalts after they solidified in the maria basins. The present absence of a lunar dipole field can be explained⁶ by showing that the disseminated radioactivity within the Moon heated the deep interior above the Curie point of iron (780°C) between 3.2 Gyr ago and the present.

Russell *et al*² have concluded from their finding of so small a magnetic dipole that this theory⁶ is highly improbable, for they suppose that it is unlikely that all traces of the ancient field could have vanished so completely. They prefer to invoke dynamo action in the lunar core, which could now have ceased generating. They have calculated² the thicknesses of hypothetical spherical shells of uniform magnetisation necessary to explain the observed moment: 300 m for a remanent magnetisation intensity of $10^{-4} \text{ gauss cm}^3 \text{ g}^{-1}$ (that of many returned samples), 11 m if an intensity equal to that for the deep interior on the theory⁶ outlined here is assumed, taking

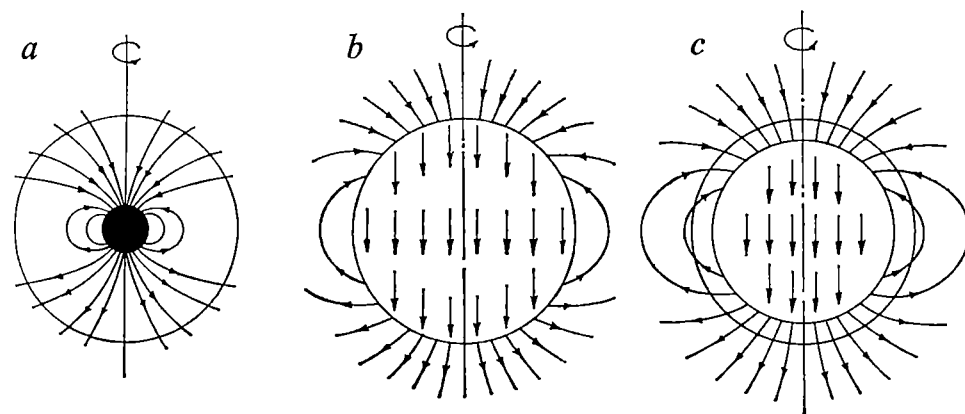


Fig 1 The ancient lunar magnetic field: a, On the core dynamo theory, b, on the primaeval magnetisation theory 4.6 Gyr ago, c, on the primaeval magnetisation theory at the time of magnetisation of the Apollo rocks, after the outer shell has been heated above the Curie point.

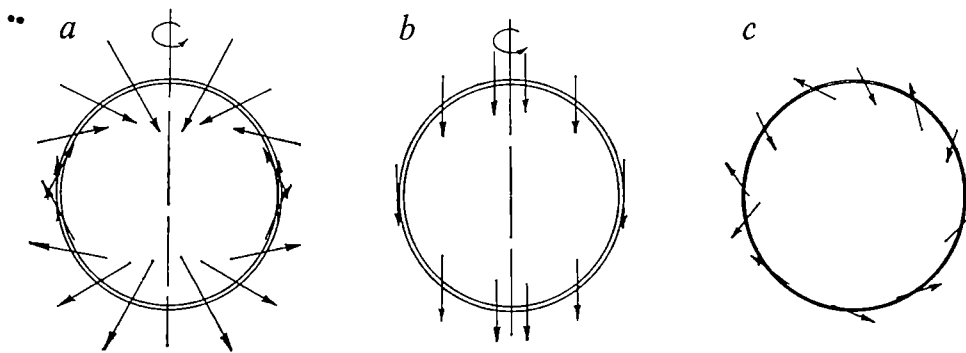


Fig 2 Models of the magnetisation of the lunar crust, *a*, uniformly magnetised crust produced by an external magnetic field, *b*, as magnetised by an axial dipole field either of core dynamo or primaeval magnetisation origin, *c*, magnetisation in a very thin shell produced locally by impacts

the value of the palaeofield (1.2 gauss at 3.9 Gyr ago) determined by Stephenson *et al.*⁷ They concluded that these thicknesses are implausibly small.

But the temperature rose to about 1,000°C in the outer parts of the Moon, say to a depth of 200 km, very soon after its origin 4.6 Gyr ago, the highlands forming by differentiation. Subsequent heating of an outer shell of the Moon to about 1,200°C between 3.9 Gyr and 3.2 Gyr must be postulated to account for the production of the magma which then flooded out into the great impact basins formed about 4.1 Gyr ago. The heat sources for these events are still the subject of debate, but electromagnetic and gravitational sources were available; these would not have heated the deep interior of the Moon in the first 1.5 Gyr, when the remanent magnetisation of the Apollo rocks was acquired, but the initial primordial magnetisation of the outer 300 km of the Moon would by then have been removed completely (Fig. 1c).

Thus, on neither theory of an internal lunar magnetic field could even a minute part of the original, general lunar magnetic field be expected to exist today: such fields as now exist must originate in the remanent magnetisation of those rocks which lie above the Curie point isotherm, say, 100 km deep.

In their calculations Russell *et al.*² assumed the model shown in Fig. 2b whereas the magnetisation of the outer parts of the Moon, acquired on the formation of the highlands and mare lavas—and possibly of deeper parts of the crust as they cooled after the major thermal events—will be directed along the dipole field directions shown in Fig. 2a. It is known that the intensity of thermoremanent magnetisation is proportional to field strength for small fields. Thus, the intensity of magnetisation of the spherical shell is $-c\nabla V$ where $c \sim 10^{-4}$ (assumed constant) and V is the magnetostatic potential of the magnetising field. The vectorial dipole moment \mathbf{M} of the magnetised spherical shell is given by

$$\mathbf{M} = -c \int_V \nabla V dv, \text{ where } dv \text{ is a volume element}$$

$$= -c \int_a \nabla V da, \text{ where } da \text{ is a surface element and } \mathbf{n} \text{ is the unit}$$

outward vector normal to the element at (r, θ, ϕ)

If the magnetising field is a centred axial dipole of moment p ,

$$\mathbf{M} = -c \int \int \left[\frac{p \cos \theta}{r^2} \right] \mathbf{n} r^2 \sin \theta d\theta d\phi$$

$$= -\frac{c}{2} \left[\int_1 \int p \sin 2\theta \mathbf{n}_1 d\theta d\phi + \int_2 \int p \sin 2\theta \mathbf{n}_2 d\theta d\phi \right]$$

where suffixes 1, 2 mean the integral is taken over the outer and

inner surfaces of the shell, respectively. As \mathbf{n}_1 and \mathbf{n}_2 at corresponding points (θ, ϕ) are opposite in sign it follows that

$$\mathbf{M} = 0$$

If the magnetising field potential is of the form $S_n(\theta, \phi)/r^{n+1}$ where $S_n(\theta, \phi)$ is a surface harmonic of degree, n , resulting from an internal source, or $r^n S_n(\theta, \phi)$ resulting from a source external to the Moon, then $\mathbf{M} = 0$ as the integrals over the two surfaces vanish separately, except that in the latter case, if $n = 1$, $\mathbf{M} = c\mathbf{v}\mathbf{H}$ where v is the volume of the shell and \mathbf{H} is the uniform field produced by an outside source, such as the Earth or Sun.

Thus, for a shell of any thickness magnetised by a dipole at its centre, the latter field being subsequently removed (Fig. 2a), the shell gives rise to zero dipole moment, because the polar regions, as shown are exactly compensated by the greater volume of more weakly oppositely magnetised material in the equatorial regions (Fig. 2a). I have also shown that all harmonics of the field external to the shell completely vanish.⁸

Thus, it does not follow from the new² limit to the dipole moment that a negligible thickness of the lunar crust is magnetised. Had this been so it may have been possible to explain the magnetisation by appealing to a yet unclarified process involving stresses in the surface rocks produced by meteoritic impact and the transient magnetic field of the solar wind locally amplified by vaporisation^{9,10}. This magnetisation would be random and confined to an exceedingly thin shell (Fig. 2c). But if that is accepted it becomes exceedingly difficult to explain the magnetic anomalies of 1γ, determined by the Apollo 15 subsatellite¹¹, the 'magcons' inferred from disturbances recorded by the Explorer 35 satellite¹². Such anomalies will result from the large craters in the magnetised crustal layers¹³, but as they arise from the leakage of lines of magnetic field from the edges of major topographical features, the effects would not be seen at heights of 100 km if the crust were only magnetised to a depth of 300 m. Russell *et al.*¹⁴ postulated instead that there are thick 'islands' of magnetised material in the basins, but their origin is obscure. I conclude that the small magnetic fields observed outside the Moon arise from the departure of the magnetised shell from the idealised model, caused particularly by the large craters.

Sceptics may wonder if the subsatellite magnetometer experiment has not simply proved the truth of a simple—but apparently previously unknown—theorem about potentials. I think, however, that the importance of the null result of the search for a present lunar magnetic dipole field is that it gives strong support to the theory that the natural remanent magnetisation of the lunar rocks requires the existence in the early history of the Moon of a magnetic dipole field of internal origin. The alternative hypothesis that the field was of external origin has been widely canvassed¹⁰. The possibility that this was the terrestrial field has been considered^{3,5} but was rejected on the grounds that the (tidal interaction) dynamics of the Earth–Moon

system precludes the Moon being within the magnetosphere for a long enough time to explain the magnetisation of the Apollo rocks. There are also arguments^{3,5} against an external field of solar origin being the magnetising agent. But the early history of the Earth-Moon system is uncertain and the magnetic fields of the early Sun and solar wind are unknown, so that new evidence² which excludes an external magnetic field as the magnetising agent of the lunar rocks is a major advance.

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Effect of thermal metamorphic conditions on mineralogy and trace element retention in the Allende meteorite

HEATING carbonaceous chondrite from the Allende meteorite in a low pressure environment causes visible mineralogical alteration at 700-1,000°C but not at $T \leq 600^\circ\text{C}$. Samples heated for 29 d at 500°C lose trace elements (Bi, In and Tl) more effectively than those similarly heated for 7 d. At 1,000°C with $\sim 10^{-5}$ atm initial pressure of O_2 , H_2 or He these elements, Ga and Se are comparatively more completely lost.

Chondritic meteorites provide valuable information about the condensation and early evolutionary histories of solid objects in the inner Solar System. Primitive chondrites probably reflect to some degree the condensation process(es) of solid material from the gaseous nebula and are particularly important in these respects. We take such chondrites¹ as comprising grades 1-4 of the accepted chemical-petrologic classification². These chondrites differ from their congeners of grades 5 and 6 in many respects

including their higher contents of volatiles, such as the trace elements Bi, In and Tl (refs 3-10) postulated as cosmothermometers⁹⁻¹². These differences could be primary (chiefly arising from differences in condensation history⁹⁻¹⁴) or secondary (chiefly arising from thermal alteration of appropriate primitive chondritic material¹⁵⁻¹⁹). Both processes probably occurred and it is desirable to evaluate the relative importance of each in evolving primitive material.

Until recently there had been no empirical attempts to determine effects on trace elements of simulated metamorphism (artificial annealing) of any primitive chondritic material. In carrying out such a study by heating samples of Allende C3 chondrite at 100°C increments over a reasonable metamorphic temperature range (400-1,000°C) in a low pressure environment (initially $\sim 10^{-5}$ atm H_2) we found substantial changes¹. After 1 week we observed minor loss of Ga and Se and major loss of the postulated cosmothermometric trace elements, but no loss of Co. This heating time is short compared with geological time, nevertheless two-element correlation patterns involving Bi, In and Tl retained in heated Allende samples are similar to those for E3-6 chondrites¹, indicating possible metamorphic effects in enstatite chondrites.

As there is no consensus^{8-11,15-19} on ambient meteoritic metamorphic environments, quite probably our experiments do not duplicate effective conditions in meteoritic parent bodies or proto-planets. As a tentative step in the direction of establishing differences in trace element retention arising from variations in ambient conditions we have conducted similar experiments on Allende samples using O_2 or He rather than H_2 and also extended the heating period using H_2 . These gases were chosen because of their very different chemical properties and their high cosmic abundances. Here we report these results together with a mineralogical and petrographic description of the heated samples.

Each heating run included chips (from a large individual, USNM-3643) and aliquots of a single batch of <100-mesh homogenised powder. We placed samples (350 mg) of powder and 2-4 chips (for trace element analysis), a 300 mg chip (for mineralogy and petrology) and 100 mg of powder (a reserve sample) into separate quartz vials which we sealed on to the precleaned heating apparatus¹. After evacuation, the system was purged with the appropriate gas (H_2 , He or O_2), re-evacuated to $\sim 10^{-5}$ atm and sealed. The end with the samples was then placed into a muffle furnace preheated to 500° or 1,000°C (Table 1). Condensable material liberated during heating was collected in a trap cooled with liquid nitrogen, the ambient gas pressure (as monitored with an ion gauge) jumped to ≥ 1 torr immediately on insertion of the apparatus into the muffle furnace but within 6-17 h the pressure reached a value which remained constant throughout the remainder of the run. After seven or 29 d we removed the sample assembly, quenched the vials in H_2O and sealed them off.

Table 1 Trace element contents of unheated and heated Allende samples

Heating temperature (° C)	Heating time (d)	Ambient atmosphere			Sample form*	Trace element contents					
		Initial gas	Initial pressure (mtorr)	Final pressure (mtorr)		Co (p p m)	Ga (p p m)	Se (p p m)	Bi (10 ⁻³ p p m)	Tl (10 ⁻³ p p m)	In (10 ⁻³ p p m)
unheated	—	—	—	—	powder	612 ± 10†	6.27 ± 0.30†	9.34 ± 0.16†	49.0 ± 0.7†	59.6 ± 2.2†	35.6 ± 1.0†
500	7	H ₂	30	175	powder	642‡	—	9.2‡	30.2‡	23‡	37.3‡
					chips	650‡	—	9.2‡	35.7‡	34‡	37.9‡
500	29	H ₂	1	36	powder	630	6.3	9.5	20.6	11	30.4
					chips	629	6.2	9.6	25.5	14	31.6
1,000	7	H ₂	20	320	powder	624‡	5.7‡	7.9‡	6.13‡	4.3‡	3.67‡
					chips	602‡	5.9‡	8.1‡	7.66‡	6.1‡	4.15‡
1,000	7	O ₂	5	240	powder	615	6.2	9.2	49.5	13.8	6.49
					chips	620	6.3	9.4	—	28.4	14.1
1,000	7	He	5	200	powder	630	4.8	5.1	10.3	2.3	≤ 4.7
					chips	651	6.1	9.6	26.7	2.4	6.10

* Powder samples were aliquots of a single homogenised batch, chips were taken from a large individual.

† Mean values of five determinations. Uncertainties listed are one estimated standard deviation from the mean calculated from the dispersion of the individual measurements¹.

‡ Data reported elsewhere¹.

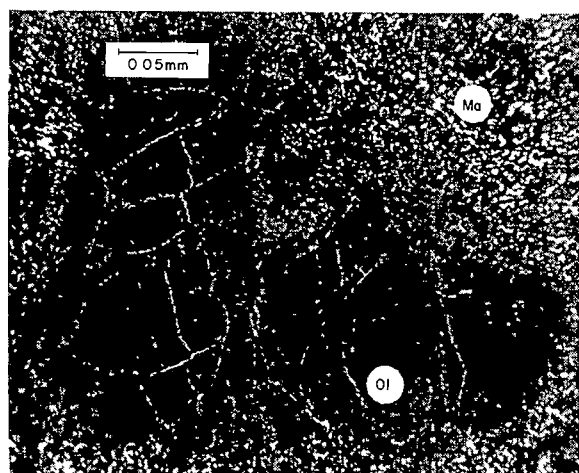


Fig. 1 Reflected light photomicrograph of Allende meteorite heated at 1,000°C, initially in H_2 atmosphere. Metallic iron particles rich in nickel are evident in matrix (Ma) and in fractures in the large olivine (Ol) crystal. Samples heated at 1,000°C, initially in He or O_2 atmosphere, seem essentially identical, implying insufficient O_2 to oxidise much of the metal formed by sulphide decomposition.

Heated samples for mineralogy and petrology and an unheated sample were prepared as polished thin sections and examined under transmitted light and reflected light. In addition, compositions of the mineral phases in several of the sections were determined by electron microprobe. We irradiated samples for trace element analysis in the Argonne CP-5 reactor for 1 week and processed them to separate Bi, Co, Ga, In, Se and Tl which were counted as described elsewhere¹. R. N. Clayton determined the $^{18}O/^{16}O$ ratios in many of our reserve powder samples and concluded that these have not been altered measurably by our heat treatment.

The samples studied, their oxygen content (in weight per cent) and the $\delta^{18}O$ (SMOW) (per mille) are: unheated (35.1) 1.1, another Allende whole rock sample (36.0) 1.5, 500°C (H_2) 29 d (34.6) 1.4, 600°C (H_2) 7 d (34.5) 1.4, 900°C (H_2) 7 d (34.0) 1.2, 1,000°C (H_2) 7 d (34.6) 1.2, 1,000°C (O_2) 7 d (34.1) 1.5, 1,000°C (He) 7 d (34.6) 1.4.

Using transmitted light we observed no visible recrystallisation effects for the transparent minerals in any of the heated samples. In all cases we found sharp crystal boundaries and well preserved textures identical to those in unheated samples. We also tried to observe effects resulting from Fe^{2+} diffusion within and between olivine and pyroxene grains. Because of the small sizes of the heated samples (~ 3 –4 mm) and the variability of the Allende samples in general, we could obtain no meaningful data for parameters such as mean iron content or percentage mean deviations (see refs 18, 19). Qualitatively, however, there was no indication of olivine or pyroxene compositions becoming more homogeneous—primary zoning was present and seemingly unaltered even for the 1,000°C run. In addition, we looked for and did not find evidence of Fe^{2+} diffusion into Fe-deficient olivine ($Fa < 1$ –2) as a result of our heating experiments. In particular, where Fe-deficient interiors of broken, zoned olivine crystals were adjacent to Fe-rich matrix there was no evidence of a diffusion profile of Fe^{2+} into the olivine.

In reflected light the unheated sample and those heated to 400°–600°C were essentially identical, but samples heated at 700°–1,000°C were markedly different. In those samples we observed no sulphide, originally present mainly as pentlandite— $(Fe,Ni)_9S_8$ —and as troilite— FeS (ref. 20). Instead we observed disseminated metal. This metal is particularly well developed in the samples heated at 900°C and 1,000°C (irrespective of

original ambient atmosphere all three 1,000°C samples seem identical after heating), occurring both disseminated throughout the matrix and along fractures in larger silicate crystals (Fig. 1). Microprobe analyses of the olivine ($Fa \sim 8$) adjacent to metal-containing fractures in the grain shown in Fig. 1 showed no decrease in Fe^{2+} content toward the fracture, indicating that the metal did not form locally by reduction of Fe^{2+} from the adjacent silicate. Instead, the metal apparently migrated inwards either by surface diffusion or vapour transport. The metal itself, in both matrix or fractures, contains 25–30% Ni, appropriate for derivation by decomposition of the sulphides. (If the metal in the fractures had formed by reduction of Fe^{2+} in the silicate, it should be Ni-deficient since the Ni^{2+}/Fe^{2+} ratio in meteoritic olivine and pyroxene is typically very low.)

Not surprisingly, the absence of discernable sulphides in samples heated at 700°C and above corresponds to the presence of off-white solid material (in the cold traps of these runs) which in one case at least was identified mass-spectrometrically (by M. Gay) as S_8 . Thus, between 600°C and 700°C, for the conditions prevailing in our experiments, sulphides in Allende samples seem to decompose ultimately to metal and sulphur. These are not the products expected by heating pentlandite or troilite in equilibrium with vapour at these temperatures²¹, but our system is open to loss of sulphur or volatile compounds of sulphur. This raises some question as to the applicability of our results to possible metamorphic models for primitive bodies since even the most recrystallised chondrites (such as Type 6) contain abundant troilite or other sulphides. But experiments in progress on enstatite chondrites show that some sulphides can be stable to higher temperatures ($\sim 900^\circ C$) and that trace element losses still occur at lower temperatures. This provides additional support for the idea that decomposition of sulphides is not prerequisite to the effects observed in samples from the Allende meteorite.

The absence of observable Fe^{2+} diffusion in any samples indicates that the diffusion rate for ferrous iron in olivine and pyroxene at the temperatures of our experiments is too slow to be observable over periods as short as a week. But this does not preclude Fe^{2+} diffusion being the principal mechanism by which uniform olivine and pyroxene compositions are produced in equilibrated ordinary chondrites^{18,19} since presumably much longer times were available.

Since the fractional loss of trace elements in Allende samples seems to depend on kinetics rather than thermodynamics¹ we were not surprised that Bi, In and Tl are lost to a greater extent from Allende samples heated for 29 d than from corresponding samples heated for 7 d at 500°C (Table 1). Relative to unheated material, In is not lost in 1 week but is lost to a minor extent by more extended heating at 500°C, Co, Ga and Se seem unaffected even in 29 d (Table 1).

The nature of the gas initially present in the heating apparatus markedly influences trace element retentivity at 1,000°C (Table 1). Relative to corresponding samples heated initially in H_2 , samples heated initially in O_2 retain the five mobile elements to a greater extent while those heated initially in He lose most of them more readily—Bi is exceptional in that it is somewhat better retained in He than in H_2 . Selenium and, more surprisingly, Bi are quantitatively retained in the O_2 case, in the He case we could establish only a conservative upper limit for In. In each case the ambient atmosphere was present at nanomolar levels, negligible compared with the amount of sample present. So it seems unlikely that differences in retentivity of the trace elements can be due to their forming or failing to form volatile chemical compounds with the gas. (Indeed, as noted, irrespective of the original atmosphere the mineralogy of the three 1,000°C samples seem identical. In particular, disseminated metal formed in all three cases indicating that the internal atmosphere could not have been extensively oxidising.) It seems more likely that both O_2 and H_2 were adsorbed on to the samples, retarding loss of the mobile elements to a greater or lesser extent. Helium would not be troublesome in this regard and indeed loss of trace elements is generally greater using this gas.

To the extent that Allende meteorite can be used as a model for other primitive materials our results have several implications relative to retention of mobile trace elements during metamorphism in primitive parent bodies. As suspected^{1,15-19} such elements can be lost by diffusion even before their host mineral(s) undergo(es) chemical or phase change. Since the duration of our experiments is short compared with geological time, our results can provide only a lower limit to the loss of mobile elements in an open system in the 'real world'. But the results clearly indicate that kinetics rather than equilibrium could play a major role in determining trace element distribution in equilibrated or partially equilibrated chondrites¹. Further, primitive meteorites differ in the amount and kind of gases or readily volatile major components they contain. Trace element retentivity also is clearly influenced by ambient atmosphere and specific regions in primitive parent bodies could conceivably be gas tight. Future experiments should allow for this possibility as well as systems completely open to gas loss. Furthermore, it seems necessary to conduct additional experiments (which are planned) for studying retentivity as a function of gas fugacity. Finally, in the event that material from other solar system objects is thermally sterilised before return to earth²² it will be necessary to choose ambient temperature and atmospheric conditions carefully so as not to degrade the sample unnecessarily. By that time we will have considerably more information on the response of other sorts of primitive material to thermal alteration.

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The Laschamp geomagnetic 'event'

SHORT lived geomagnetic 'events' provide useful stratigraphic markers, they may enhance climatic and evolutionary changes^{1,2} and they provide stringent parameters for geomagnetic models. During such events the virtual geomagnetic pole undergoes excursions outside the usual range of secular variation but they are usually brief, generally lasting about 10^5 yr, so that their

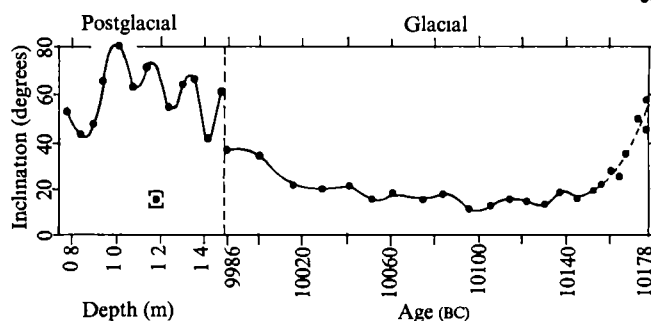


Fig. 1 Inclination of remanence after demagnetisation in an alternating field for samples from the Starno core. The rapid change in inclination before 10160 BC reflects the influence of water currents on the depositional remanence, the currents diminish rapidly as a consequence of glacial recession.

magnetic record in deep sea sediments is often lost because of post-depositional remagnetisation³ and because of biological activity in the upper few decimetres of the sediments⁴. In lake sediments, where sedimentation is more rapid, the magnetic record is better preserved. Dating is, however, difficult because secular variations in atmospheric carbon isotopes and irregular distributions in the environment are known to produce errors. Furthermore, geomagnetic events, the levels of which may have been displaced by bioturbation⁴, are usually dated by assuming uniform sedimentation between ¹⁴C dated horizons. The Laschamp event, in particular, seems to have been detected at 12 locations although the reported age (Table 1) varies between 7,000 and 17,000 yr BP, a range outside the quoted error for individual determinations, but within the realistic error in dating.

Fig. 2 Motion of the Starno virtual geomagnetic pole. Pole positions are computed from the sample inclinations (Fig. 1) and the interpolated values of declination from the long core measurement, corresponding to the age of each sample Δ , Postglacial mean direction (7800 ± 100 BC).

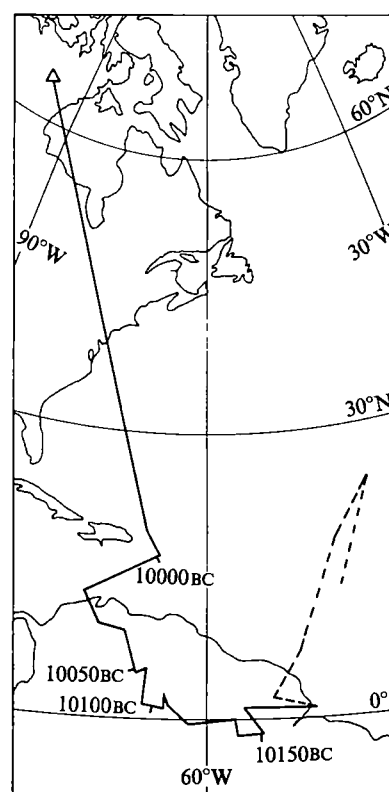


Table 1 Geomagnetic events probably corresponding to the Laschamp Event

Locality	Age (bc)	Dating method	Material	Reference
Puy de Laschamp, France	7000-9000	C ¹⁴	Volcanic scoria	15
Lake Tahoe, USA	≥ 1100		Lake muds	16
Gothenberg, Sweden	> 12350	C ¹⁴	Varved clay/silt	17, 18
Viby, Sweden	12400	Varve chronology	Varved clay	6
Lake Erie, USA	≥ 12500		Lake muds	19
Mono Lake, USA	< 13300	C ¹⁴	Lake muds	20
Lake Windermere, UK	13400 ± 400	C ¹⁴	Lake muds	21
Lake Chalco, Mexico	14450	C ¹⁴	Lake muds	22
Gulf of Mexico, USA	12500-17000	Fauna	Marine muds	23
Tlapacoya, Mexico	14770 ± 280	C ¹⁴	Lake muds	24
Gulf of Mexico, USA	17000 ± 1500	Fauna	Marine muds	25
Lake Biwa, Japan	17600-18700	C ¹⁴	Lake muds	26, 27
Laschamp + Olby, France	< 20000	K/Ar	Lavas	28
Blekinge, Sweden	12077-12103 ± 150	Varve chronology	Varved clay	This work

At least four other established excursions have occurred during the last 0.69 Myr: the Biwa II and I events at 295,000 and 181,000 BP, respectively²⁶, the Blake Event at 111,000 BP²⁹, and the Mungo Event at 30,000 BP³⁰. Others undoubtedly exist as such excursions are known throughout the geological record, for example during the Palaeo-Eocene³¹. Another event in silts of the Puget Lowland, USA was originally considered to be 20,000 yr old, but is now thought to be older than 45,000 BP (K. L. Othberg, personal communication) and is, therefore, more likely to record the Mungo rather than the Laschamp Event.

In order to determine the age and duration of the Laschamp event we obtained samples of Swedish Quaternary laminated clays using the Swedish Geological Survey foil corer⁵, whereby undisturbed, oriented cores 3.6 cm in diameter and up to 11 m in length can be obtained. Varved sediments, which have not undergone bioturbation, are particularly suitable for palaeomagnetic investigations, and have been studied in Sweden^{6,7} and in North America⁸. Those studies involved measurements on individual samples, collected separately or extracted from cores.

After preliminary results had been obtained from a Swedish Geological Survey unoriented sediment core, new oriented cores were taken from two sites in southern Sweden (Blekinge), at Stilleryd (56°18'N, 14°83'E) and Starno (56°16'N, 14°85'E). Each core comprised a lower, varved, glacial sequence and an upper, postglacial, unvarved deposit. The susceptibility and natural remanence of each core were measured⁹ at intervals of 1 or 2 cm and then samples of characteristic lithology were selected from both sites to test their stability in alternating magnetic fields. The results indicated that the samples were moderately stable up to at least 600 oersted. The cores were then demagnetised in fields of 100 or 150 oersted. The remanence of the cores was interpolated to the positions of the inter-varve boundaries using cubic spline functions in order to adjust the data for changes in the rate of sedimentation. Age bounds for the postglacial sequences were fixed by relating the altitude of each site and its environs to the shorelevel displacement curve for eastern Blekinge¹⁰.

Each core was then cut into lengths of 5 or 6 cm and the samples were measured on a Digico magnetometer¹¹. The inclinations of remanence in the Stilleryd core which showed post-depositional chemical alteration of the sediment were found to be scattered, with no obvious trends. At Starno, the record of inclination has been preserved (Fig. 1), with a marked magnetic unconformity at the base of the postglacial deposit. The varves of that sequence have been correlated with those at Stilleryd and an absolute date has been determined by connection with a series in the Swedish chronology. This is generally considered¹² to be more accurate than radiocarbon dating and is probably correct to ±150 yr (absolute) in the series considered here. The relative ages within each section are probably correct to within 5 yr. Measurements of the remanence immediately beneath two slumps in the Stilleryd core indicate that the magnetisation became blocked less than 9 yr after deposition and so the ages of sedimentation and magnetisation are virtually coincident in the undisturbed varve sequences.

We have computed the path of the virtual geomagnetic pole corresponding to the Starno palaeomagnetic record (Fig. 2). It indicates a geomagnetic quasi-reversal between 10153 BC

and 10127 BC followed by a northward migration of the virtual pole at a rate of about 2.8° of latitude every 100 yr. The southernmost excursion of the pole lies close to the meridian 60°W, 120°E, found by Steinhauser and Vincenz¹³ to be one of two preferred paths for palaeopoles during polarity transitions.

Studies of the unvarved sequences indicate anomalous changes in declination and low inclination values around 860 BC. This may well represent a more recent geomagnetic event, the Starno event, and may be the reversal reported by Ransom¹⁴ on the basis of archaeomagnetic studies by Folgheraiter and Mercanton on contemporary fired clay and pottery, from Greece and Bavaria. At this stage, however, the event is defined in non-varved sediments and we intend to examine the corresponding horizon within a varved sequence to determine the age and nature of this more recent excursion.

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Alternative to the geomagnetic self-reversing dynamo

A PRIMARY task of geomagnetism is to account for the magnetic field of the Earth and for the occasional reversals of that field. Present research in geomagnetic dynamo theory aims to develop a physically realistic, mathematically solvable, self-reversing dynamo model¹—one that contains the seeds of its own reversal. Here I point out that there is an alternative method of analysing the geomagnetic field which can account for magnetic reversals but which does not require a self-reversing dynamo. The analysis is based on the assumption that the geomagnetic field arises from two separate sources. Each source has a mathematical representation in terms of dipoles, quadrupoles, and so on. The magnetic field which is observed at or above the surface of the Earth is the sum of the fields arising from the two sources. In particular, the observed dipole component of the Earth's field is the vector sum of the dipole components of each source.

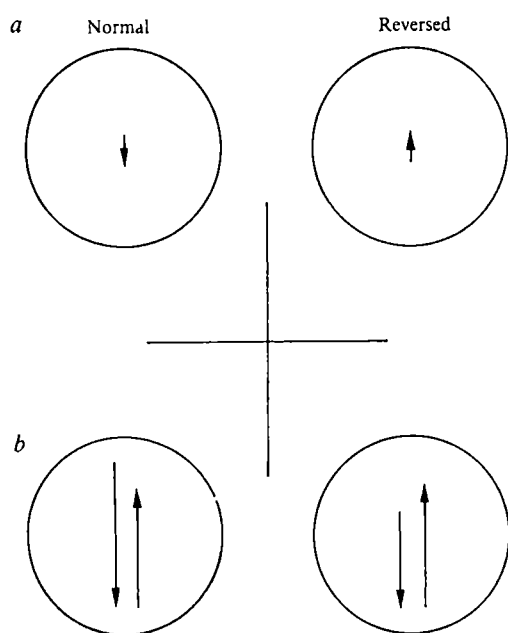
$$\mathbf{M}_0 = \mathbf{M}_1 + \mathbf{M}_2 \quad (1)$$

If the dipole components of each source are oppositely directed, one pointing essentially towards the north rotational pole and the other essentially towards the south rotational pole, then

$$\mathbf{M}_0 \simeq \mathbf{M}_1 - \mathbf{M}_2 \quad (2)$$

The prevailing magnetic polarity reflects the polarity of the dominant source component (Fig. 1). A magnetic reversal, which corresponds to a change in the sign of \mathbf{M}_0 , represents

Fig. 1 Schematic diagram of polarity states. *a*, States of a self-reversing dynamo; *b*, states based on a two-source model. Net field is the same in both cases.



a shift in the relative sizes of \mathbf{M}_1 and \mathbf{M}_2 . If both \mathbf{M}_1 and \mathbf{M}_2 are very large compared with \mathbf{M}_0 , then only small fluctuations in \mathbf{M}_1 , \mathbf{M}_2 , or both will result in a reversal.

The foregoing analysis is appealing in that it avoids the complexities inherent in a self-reversing dynamo theory. First, a self-reversal can not arise from a steady-state solution so that the full time-dependent solutions must be obtained for any given model. Rikitake² was able to demonstrate that a highly idealised double-disk dynamo showed time-dependent behaviour which resulted in self-reversal. Subsequent work, however, has been severely hampered by the complexity of the magnetohydrodynamic fluid motions and the intractability of the associated equations. In the proposed model, a full time-dependent treatment is not necessary since one or both sources need only exhibit (small) fluctuations in magnitude when subjected to (small) perturbations.

Second, there is no need to treat the physical and thermal consequences which result from the large scale conversion of magnetic energy into kinetic energy and back to magnetic energy occurring during a self-reversal³. But mathematical simplicity, in itself, is not admissible as evidence for a particular model; the observed data must be consistent with a two-source analysis and a plausible explanation for the two sources must be given.

If the Earth's field arises from two sources, then differences are to be expected between the normal and reversed polarity states, indicative of the fact that a different source is dominant in each state. Two such effects have been observed. Wilson⁴ grouped palaeomagnetic data by polarity and discovered significant differences in the time-averaged mean pole positions of the normal and reversed populations. Dagley and Lawley⁵ reviewed reported studies of transitional behaviour of the geomagnetic field and found a clear predominance of reversed over normal transitions, which led them to conclude that the reversed state is less stable than the normal state. A recent statistical study of the observed sequences of reversals by Phillips and Cox⁶ came to the same conclusion. Thus, systematic asymmetries may well exist between the two polarity states of the magnetic field.

Analysis of the present field may also reveal whether the Earth's field has two sources. I have developed⁷ a technique for fitting a set of spherical harmonic coefficients with a combination of geocentric axial dipole and eccentric (not necessarily axial) dipole. The free parameters are the relative strengths of the two dipoles as well as the coordinates of the eccentric dipole. When applied to the first eight coefficients of the International Geomagnetic Reference Field of 1965, the best fit consists not of two more or less parallel components whose sum is the present field, but rather two large, more or less anti-parallel components whose difference is the Earth's field (Fig. 2). This is precisely the configuration described in equation (2), so I conclude that there is some geomagnetic and palaeomagnetic evidence consistent with a two-source model.

As for an explanation of the two sources, the obvious possibilities are the inner and outer core. The source field from the outer core would still arise from a magnetohydrodynamic dynamo since the liquid nature of the outer core is well established. As noted above, steady-state solutions which exhibited fluctuations in magnitude would be adequate.

The central question is then whether the inner core can be the source of a magnetic field. That there exists a distinct inner core is now well established. The structure at the inner core-outer core interface has been mapped in considerable detail⁸. Recent observation of the propagation of shear waves through the inner core confirms that it is in a solid phase⁹. Clearly, a magnetohydrodynamic system is inappropriate for the generation of a magnetic field in the solid, inner core. A mechanism, if it exists, must come from solid state physics. A permanent magnetisation of the inner core resulting from ferromagnetism is not possible if the inner core is above its Curie temperature. Although this is probably the case, the theoretical extrapolations needed to conclude that the core is above its Curie temperature have never been tested experimentally at the pressures

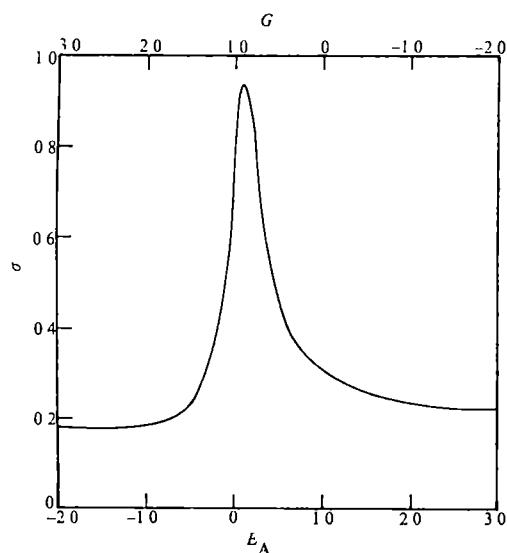


Fig 2 Fit of the IGRF field with eccentric dipole and geocentric axial dipole. Parameter G is mean square deviation in arbitrary units E_A is the magnitude and direction of the axial component of the eccentric dipole G is the magnitude and direction of the geocentric axial dipole. The vector sum of E_A and G is 1.0, the normalised, net axial component

and temperatures of the inner core, so it is not known for certain whether the core is above its Curie temperature. Indeed, all thermodynamic parameters for the core must be based on extrapolation. Even the question of composition has not been completely resolved. In addition to iron, the inner core may contain large amounts of nickel and small amounts of carbon, sulphur and silicon¹⁰. Given this situation we can only conclude that the magnetic and electrical (semiconducting?) properties of the solid inner core have yet to be fully explored. So it is possible that at the particular and extreme pressures and temperatures of the core there may be new cooperative solid-state phenomena which could lead to a permanent magnetisation. Such a suggestion is not without precedent. In the case of hydrogen, Ashcroft¹¹ has shown theoretically that at high temperatures and pressures, superconductivity may occur.

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Is there an Icelandic mantle plume?

SCHILLING¹ has interpreted the chemistry of basalt lavas from the Reykjanes Ridge and Iceland, identifying two distinct upper mantle sources for these lavas, one of which rises in a primordial hot mantle plume beneath Iceland. I questioned² this interpretation on the grounds that insufficient attention had been given to the possibility that the magmas were derived from a homogeneous source, the differences could arise from by fractional crystallisation during the ascent of the lava

Schilling³ pointed out two factual errors in my comments, which I accept. But his other comments reveal a misunderstanding of my ideas on the nature and effects of olivine-gabbro and eclogite fractionation. The facts of the distribution of the rare-earth elements (REE) as revealed by Schilling (ref 1, Table 1), conflict with later statements³, they are, however, consistent with variable fractional crystallisation of lavas from a common source, or even from a source which is more depleted beneath the ridge.

Schilling's statements³ on primary magmas, the role of fractional crystallisation, and the significance of phenocrysts, seem to conflict with normally accepted models, and lead to constraints concerning upper mantle mineralogy and chemistry which were not among the many geophysical-geochemical requirements considered in setting up his model.

Basalts richest in REE do not show the depletion in the lighter REE which I formerly (incorrectly) expected² to result from significant fractionation of clinopyroxene (accompanied by olivine and plagioclase). There is now no impediment to hypotheses which involve clinopyroxene fractionation.

Schilling³ deduced that a high clinopyroxene-garnet ratio (~95/5) is necessary in any eclogite cumulus if the constraints of the rare earth element (REE) pattern are to be met. At a pressure of 30 kbar the coprecipitation ratio of clinopyroxene to garnet seems likely to be close to 57/43 (ref 5), the same ratio at 40 kbar seems to be closer to 86/14 (ref 6). The high clinopyroxene-garnet ratio in the extract is a result of the high solubility of Al_2O_3 in the pyroxene Al_2SiO_5 , which at lower temperatures or other pressures would crystallise or exsolve as garnet, will be present as clinopyroxene in the actual cumulates which form from, and partition REE against, the liquid. The bulk extract still resembles the coexisting liquid in major chemistry, and massive extraction of an eclogite of this type would not necessarily lead to a major element chemistry distinct from that of the forerunners of the Reykjanes Peninsula olivine tholeiites (see ref 5).

Schilling³ defined eclogite fractionation as "clinopyroxene-garnet extraction in a 50-50% mixture", implying that this ratio is required by O'Hara and Yoder's⁵ eclogite fractionation model. It is not.

Schilling³ challenged my statement² that there was massive relative depletion of heavy REE in the more northerly basalts. The La/Yb ratio is of the order of 0.28 in the more southerly basalts and of the order of 2.04 in the more northerly basalts. Relative to La, Yb in the more northerly basalts has been depleted to 0.14 of its abundance in the more southerly basalts, I consider that to be a massive relative depletion. The average Yb contents (from ref 1, Table 1) of 13 samples taken south of 62°N are 3.0 p.p.m., and those of 20 samples taken north of 63°N are also 3.00 p.p.m., for 9 samples taken north of 63°N they average 2.0 p.p.m. There may, therefore, even be absolute depletion in Yb on passing north to Iceland, since the fall exceeds the $\pm 15\%$ precision quoted³. Schilling's (ref 3, Fig 1b) own graphical presentation demonstrates the validity of the very remarks which he himself says are wrong³.

I identified the upper drawing of Schilling's³ Fig 1 as Fig 1b and the lower drawing as Fig 1a and I assumed that it is the basalts collected between 63°N and Iceland which are symbolised by the triangles.

In that case, Fig 1b (ref 3) demonstrates that the observed trend of $(Yb)_{EF}$ against $(La/Sm)_{EF}$ could be a result of eclogite fractionation at depth followed by olivine-gabbro fractionation near the surface, with the extent of fractionation increasing northwards towards Iceland. This strongly supports my model in which "magmas rising through this thicker (Icelandic) superstructure take much longer periods between formation and the eruption allowing greater scope for fractional crystallisation at all pressures during ascent". Naturally, further south from 60°N, there will be little further change in $(La/Sm)_{EF}$ unless the thickness of volcanic superstructures and the consequent hydrostatic head of magma decreases markedly, which they do not so far as is known.

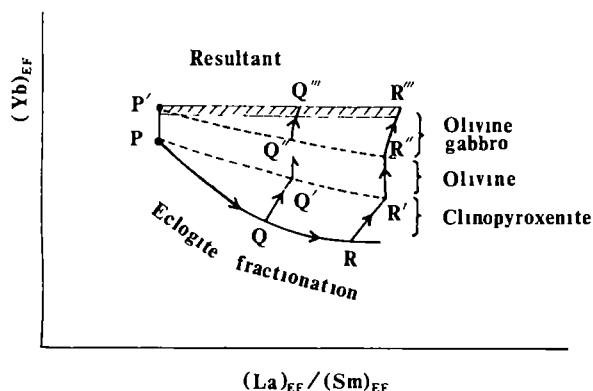


Fig 1 Variation in the enrichment factor of Yb against the relative change in the enrichment factors of La and Sm during fractionation of REE. Vectors are proportional to the amount of fractionation undergone in different pressure regimes. The figure illustrates how the flat trend observed can be the result of the fractional crystallisation proposed. P, primary magma, P-P', fractionation path to erupted ridge magma, P-Q-R-R'-R''-R''', suggested fractionation path leading to Icelandic basalt.

Schilling³ now reports that $(La/Sm)_{EF}$ does not, in fact, decrease significantly on passing to the south along the ridge.

I have here considered only the effects of eclogite fractionation at high pressures and olivine gabbro fractionation at low pressures, but my model logically calls for fractionation in an intermediate pressure regime also, where crystal extracts are likely to be dominated by the separation of olivine and clinopyroxene (as in Yoder and Tilley's⁷ stage of pyroxene fractionation). Figure 1 shows the nature of the proposed effects in the $(Yb)_{EF}$ against $(La/Sm)_{EF}$ plot. The possible influence of the participation of spinel in the crystal liquid equilibria should not be overlooked. Spinel could be expected to retain lighter REE.

Implicit in Fig 1 is the concept that totally incompatible elements in erupted basalts may show enrichment factors (relative to their primary magma) ranging from 2 to 20, and differential enrichment factors as high as 10. Hart *et al.*⁸ clearly consider this to be unreasonable, but such a model is indicated by data for both the REE and the lithophile elements with large ions. Eclogite fractionation at high pressures does, moreover, afford the possibility that some K could be extracted (in omphacite) with Rb remaining excluded, that would lower the K-Rb ratio of the residual liquids (in accordance again with observed differences between Icelandic and ridge basalts).

The main source of our divergence of views may be the differing enthusiasm with which Schilling and I search for ways of explaining the minor and trace element chemistry, any explanations must, of course, be simultaneously compatible with current geodynamic hypotheses and with the major element chemistry which indicates that extensive fractional crystallisation is a common process in the history of erupted magmas. For example, changes in the nickel contents of basaltic liquids are controlled by the very high distribution coefficient for this element in favour of coexisting olivine and spinel crystals. But if all the rocks studied have suffered comparable fractionations of these minerals, there will be no wide divergence of Ni content. Eclogite fractionation, in contrast, has a much smaller effect on the Ni content of the residual liquids⁵. The combination of relatively small variations of Ni content with large variations in incompatible element concentrations, far from being an impediment to the proposed mode of eclogite fractionation⁸ is a fulfilment of another prediction for the eclogite fractionation model.

It is obvious from Schilling's¹ Table 1 that the large degree of differential fractional crystallisation implied by my Fig 1 cannot be correct unless either, first, K_2O and P_2O_5 do not

behave as entirely incompatible elements (there is the possibility of loss of a fugitive phase from more fractionated basalts) or, second, the source mantle beneath Iceland is now significantly depleted in K_2O , P_2O_5 and perhaps $Fe/Fe+Mg$, relative to the mantle supplying the basalts at the ridge to the south of Iceland. The latter interpretation would be at least consistent with the observations that much more basalt has been produced in the past from the sub-Icelandic mantle. Although directly contrary to the primordial hot mantle plume hypothesis, this interpretation fits naturally into an alternative hypothesis suggested here.

Schilling referred¹ to "production of uniform primary magmas". "Primary magma" is a technical term which has been defined⁹ as a mass of magma which originated by tapping some body with a primitively liquid condition inherited from a remotely early stage in the Earth's history, or obtained by partial or complete fusion of pre-existing solid rock. That definition specifically excludes from the category of "primary magma" masses of magma modified by differentiation or contamination.

Schilling's model is specific and clearly stated^{1,4}. The sense in which Schilling³ now claims to have used the term 'primary' when discussing magmas differs from the text-book definition, which would have been confusing to igneous petrologists. Schilling's papers^{1,4}, however, show that he intended the term 'primary magmas' to be used in the same sense as that conveyed by the textbook definition.

In those papers^{1,4} the possibility of fractional crystallisation at shallow depths is discussed only in connection with its rejection as a mechanism for explaining the chemical variations, and as a cause of excess scatter over the dispersion error bars in a variation diagram (ref 1, Fig 2). Fractional crystallisation forms no part of the definitive statements of Schilling's^{1,4} model.

Rather than the model requiring fractional crystallisation near the surface to produce the observed phenocrysts, it is clear that Schilling envisages them as residual crystals from the partly melted source mantle, the mere presence of which in the lavas would necessarily exclude the possibility of fractional crystallisation.

If the phenocrysts are residual crystals from the partial melting of the respective source mantles at low pressure, four predictions would follow. First, the residual upper mantles would contain olivine, plagioclase and augite, but no orthopyroxene. Second, the fertile mantles would contain so little, if any, orthopyroxene that it would be the first major crystalline phase to be melted out during partial melting. Third, the residual mantles would contain olivines of composition Fo_{78-85} , and before melting the source mantles would have to be even less magnesian. Finally, the magmas from Iceland, derived from the primordial hot mantle plume would have to be in equilibrium with their phenocryst assemblage at a higher temperature than are the magmas from the ridge.

The first three predictions envisage an upper mantle quite different from popular models (such as those including nodules in kimberlite or basalt, orogenic peridotite masses, pyrolite (III)) in all of which orthopyroxene is prominent and remains with olivine as a residual crystal until very advanced levels of partial melting have been reached, and all of which envisage a considerably higher $Mg/Mg+Fe$ ratio in the source mantle, such that the olivines would have a composition Fo_{94-88} . The fourth prediction can be tested by experimental petrology but published data lead to the expectation² that it will be found to be untrue.

Schilling¹ has indeed listed and taken into consideration many geophysical-geochemical features in setting up his model. But among them I do not find included the geophysical consequences of low $Mg/Mg+Fe$ in the upper mantle, or the geochemical, indeed cosmochemical, consequences of lower Si/Mg and Mg/Ca ratios in the upper mantle than would be indicated from natural ultrabasic rocks or chondrite-based models of element abundances. Nor has he considered the

most fundamental geophysical objection to his hypothesis—density. Other models will have to be sought which fit all the constraints, not least among which is the fact that the erupted basalts are thoroughly fractionated at low and probably higher pressures, and cannot be used directly to characterise the geochemistry of their source regions.

Any mantle rock which is significantly more 'fertile' than another with regard to basalt production is going to be richer in garnet or spinel, and richer in Fe/Mg in its olivine and pyroxene, than less 'fertile' mantle. The more fertile mantle would therefore be appreciably denser than the less fertile mantle (by a factor of parts per hundred) and this difference could only be offset by a temperature difference of the order of thousands, rather than of tens¹, of degrees centigrade. (The orders of size used here are based on several considerations: the specific gravity of olivine increases by 0.014 g cm⁻³ for every 1 % decrease in forsterite content, if fertile mantle is the same as infertile mantle +20% potential basalt, and the potential basalt were crystallised as garnet plus pyroxene (density 3.6 g cm⁻³) distributed in the potential infertile peridotite residuum (3.3 g cm⁻³), the fertile mantle will have a density of ~2.5 parts per hundred higher than the infertile mantle. Coefficients of cubic expansion in silicates are taken to be ~10⁻⁵, not ~10⁻².)

In other words, if fertile mantle plumes exist at all, they should be sinking, not rising. It is, therefore, appropriate to consider at least two alternative models which are consistent with the relative densities of the rocks concerned.

The first retains the concepts of a plume of hot mantle material rising from great depths beneath Iceland, but supposes it to be composed of still fertile, yet more depleted mantle than is rising beneath the ridge (thus, it inverts Schilling's hypothesis). Its less fertile character would have been imposed by partial melting events which occur at the base of the upper mantle¹⁰. The resulting lower density of the partly depleted peridotite could cause it to begin to rise in localised diapirs through the more fertile and cooler overlying mantle. In spite of its less fertile character the rising diapir would nevertheless be capable of yielding abundant magma on account of its higher temperature and relatively rapid decompression. It is still necessary to suppose that magma eruption would be slower from this plume, because of the hydrostatic head, and that fractionation of the magmas occurs at all depths. An important factor would be the retention of at least some of the incompatible elements during the first partial melting events at the base of the upper mantle, caused by their incorporation in crystalline phases at very high pressures (~120–150 kbar).

An alternative to that hypothesis merely requires that a 'hot spot' starts because at some time there exists at some point a more rapid effusion of magma from the base of the upper mantle¹⁰. Following the build up of hydrostatic head in the magma once an island had been created, eclogite precipitation would begin and would lead to an increase in the density of the mantle beneath the 'hot spot'. That could lead to downsagging of the mantle beneath the 'hot spot' which in turn may be supposed to distort the stress field in such a way that the 'hot spot' continues to receive an undue share of magma from depth, thus becoming self propagating. This model does away with plume motion in the mantle and predicts a gravity high in the Iceland region.

Finally, I did not suggest that isotopes had been fractionated, but that isotope ratios had changed during the manifest fractional crystallisation. The distinction is vital.

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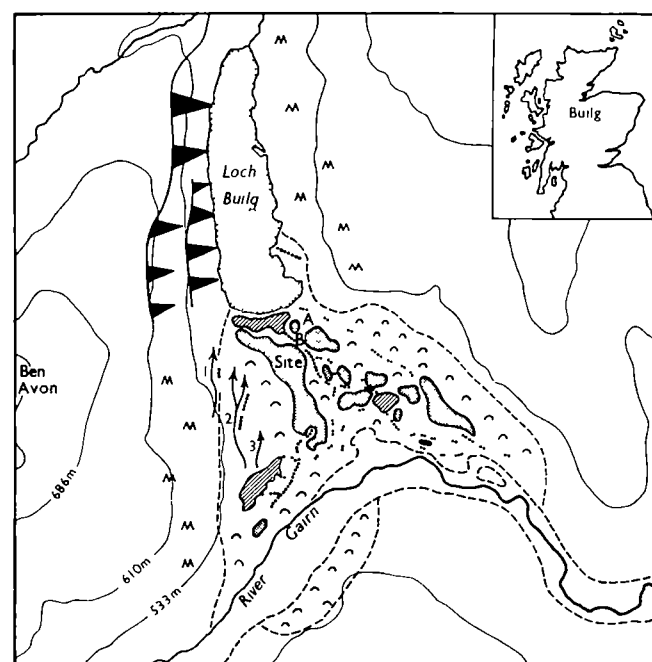
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Loch Lomond Readvance in the eastern Cairngorms

So far in the Cairngorm Mountains it has been impossible to confirm speculations about the physical extent of the glaciers of the Loch Lomond Readvance by absolute dating. As recently as 1970 it was therefore possible to argue without contradiction for anything between a full Scottish ice sheet and a handful of corrie glaciers¹. Subsequent evidence from the adjacent Spey Valley precludes the former possibility^{2,3}, and attention is now focused on the possibility that there was restricted ice cover about the time of the Loch Lomond Readvance. Evidence from the vicinity of Loch Buirg, at an altitude of 525 m in the eastern Cairngorms, throws further light on the problem.

The main feature of this area is a marked concentration of hummocky landforms at the intersection of the River Gairn valley and the glacial watershed breach containing Loch Buirg (Fig. 1). This topography is made up of steep-sided conical mounds, narrow ridges, flat-topped mounds and marshy or water-filled hollows. It has a relief amplitude of up to 20 m. The hummocks have previously been interpreted as the moraine limit of the Loch Lomond Readvance in this area^{4,5}. Field mapping strongly suggests, however, that they are a complex of kames and kettles associated with stagnant, downwasting ice of the last major ice sheet. There are few good sections exposing the internal composition of the hummocks but one of them does reveal well-sorted, crudely bedded sand and gravel and all

Fig. 1 Glaciated region around Loch Buirg, Scotland



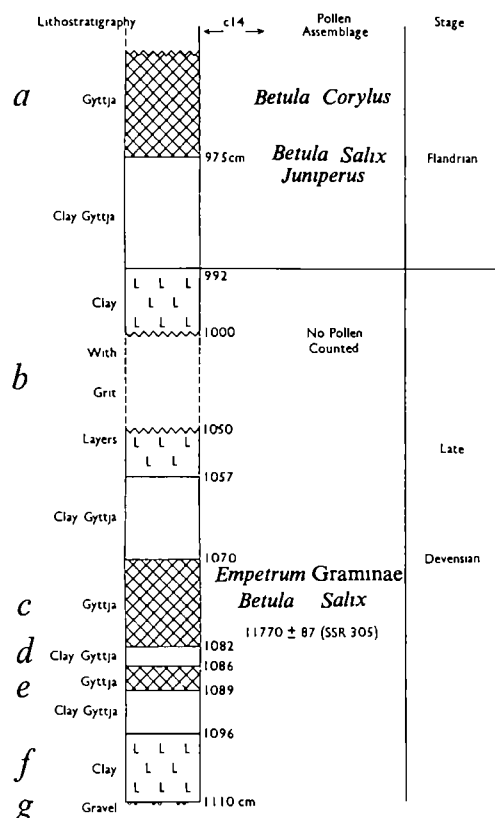


Fig 2 Stratigraphy of the basal sediments at Loch Buiig

of the superficial exposures consist of water-worn pebbles and sand. Many of the hummocks have little obvious topographic alignment but numerous eskers and meltwater channels help establish the dominant trend and probable development of the former meltwater drainage system. There are two main directions of meltwater drainage indicated by the landforms. One enters the Loch Buiig area from Glen Gairn and trends northwards into the River Avon valley, it is represented by channels 1-3 (Fig 1) and contiguous eskers, all of which lie over 15 m above the valley floor and probably represent an early phase of drainage.

The other, lower group of eskers and associated kame terraces, shows the progressive realignment of the meltwater drainage system from the northern route to a lower, eastern route down the Gairn Valley. Eskers A and B (Fig 1), for example, were probably deposited by streams that entered the western side of the Loch Buiig breach from Glen Gairn, but curved back into the Gairn Valley when the route north had become abandoned.

The stratigraphy of an infilled kettle hole just south of Loch Buiig (Ordnance Survey GR NJ 189028) has been examined using both a Hiller borer and, subsequently, a Jowsey borer. The basal sediments show features generally associated with the late Devensian stage. From 992-1,057 cm in the cores is a grey plastic clay (Fig 2b) with stones and grit layers interpreted as a solifluction deposit. Beneath this, from 1,070-1,089 cm, is a brown organic gyttja interpreted as an interstadial deposit, though there is a more complex stratigraphy than is usual, for a distinctly clayey layer (Fig 2d) at 1,082-1,086 cm, divides the gyttja into two parts (Fig 2c and e). At the base is a grey clay (Fig 2f) which gives way to gravel (Fig 2g) at about 1,110 cm. This stratigraphy has been confirmed in several borings and there is no evidence for an ice advance over the site after the start of the deposition of these sediments.

Preliminary pollen counts of 200 grains have been carried out on samples collected by a Hiller borer from different depths in the sediments. Seven samples from gyttja between

1,070 and 1,089 cm show an assemblage dominated by non-tree pollen. *Empetrum*, Graminae and Cyperaceae are most common, together with the shrubs *Salix* and *Juniperus* and open land herbs such as *Rumex*, *Artemisia*, Compositae, and Caryophyllaceae. Together, these make up 80% of the total pollen. The only tree pollen consistently present is *Betula* which comprises 10-20% of the total. This pollen assemblage is typical of the late Devensian in Scotland and similar assemblages have been recognised from the late Devensian at Abernethy³, the Allerod Interstadial at Loch Kinord, Loch of Park⁶, Garral Hill, Keith (where it has been radiocarbon dated between 10,800 and 11,880 b.p.)^{8,9} and elsewhere in northern Scotland¹⁰.

No pollen has been obtained from the clay between 992 and 1,057 cm.

In the uppermost gyttja (Fig 2a), sample counts have been carried out at widely spaced intervals. At 975 cm *Betula* pollen contributes 45% of the total land pollen, and *Juniperus* contributes 14%. *Salix*, *Empetrum* and Graminae are still important, but open land herbs have declined markedly. By 995 cm, *Betula* contributes 66% of the grains and *Corylus*, 13%. *Juniperus*, *Salix*, Graminae, *Empetrum* and the open land herbs have now greatly declined. At 900 cm *Betula* contributes 32% and *Corylus*, 58%. *Juniperus*, *Empetrum* and the open land herbs are no longer present. It seems that these pollen spectra compare well with the *Betula-Juniperus* Assemblage Zone and the *Betula-Corylus* Assemblage Zone of the early Flandrian at Abernethy³. They also compare with Zones IV and V at Loch Kinord, which again are early Flandrian⁶.

Thus, the pollen evidence suggests that the bottom deposit predates the Loch Lomond Readvance and that the clay deposit between 992 and 1,057 cm formed during the post-interstadial (Zone III) and is thus the time equivalent of the Loch Lomond Readvance.

Material for radiocarbon dating was obtained with the aid of repeated cores using a Jowsey borer. Gyttja (Fig 2c) was divided into an upper and lower half and bulked to form two samples, 1,070-1,067 cm and 1,076-1,082 cm. The bulked sample of the lower half of gyttja (1,076-1,082 cm) is dated at $11,770 \pm 87$ b.p. (SSR 305). This date confirms both the interpretation of the stratigraphy and the pollen analysis gyttja (Fig 2c and e) from 1,070-1,089 cm formed during the late Devensian interstadial and, therefore, predates the Loch Lomond Readvance.

It was not possible to obtain a sufficiently large sample of the lowest gyttja (1,086-1,089 cm), (Fig 2e) to allow radiocarbon dating, because gravel (Fig 2g) at the base prevented penetration of the point of the corer. Certain suggestions regarding its age are, however, possible. If one assumes a constant rate of deposition and an even distribution of organic material in gyttja (Fig 2c), then a composite date for the lower half of the layer would represent a date of approximately one quarter the way through the period represented by the layer. As 11,770 b.p. is approximately one quarter of the way through the Allerod *sensu stricto* (10,750-11,950 b.p.)¹⁰ it is tempting to attribute the base of gyttja (Fig 2c) to a date of approximately 12,000 b.p. Thus, it seems probable that clay-gyttja and gyttja (Fig 2d and e) may be significantly older than 12,000 b.p., though further dates are necessary to confirm or refute this. If this suggestion is confirmed then this site supports the evidence of early deglaciation in the Scottish Highlands which has already been suggested by radiocarbon dates and pollen stratigraphy from Wester Ross¹¹, the Spey Valley², the Teith Valley^{8,9}, Sutherland^{8,9} and Invernessshire^{8,9}.

The Loch Buiig results have several implications for studies of Scottish Quaternary stratigraphy. First, the geomorphological evidence suggests that the fluvio-glacial landforms in the Loch Buiig area are associated with the deglaciation of a major ice sheet and the only evidence in the vicinity for a later glacier readvance is in the corries on Ben Avon¹². This supports the interpretation previously suggested for the adjacent western Cairngorms¹.

• Second, an area close to the heart of the Cairngorm Mountains, at an altitude of 525 m, was free of its main ice sheet cover earlier than 12,000 BP

Third, the site has not been overrun by glaciers of the Loch Lomond Readvance. Thus, it is reasonable to suggest that any Loch Lomond glaciers must have been restricted to higher parts of the massif, for example the corries of Ben Avon

Fourth, the identification of the limits of the Loch Lomond Readvance by mapping the distribution of 'fresh hummocky moraine' must now be considered to be a very precarious technique. The suggestion⁵ that the Builg hummocky moraine represents the limits of a Loch Lomond glacier in the valley of the Gairn is clearly contradicted by the evidence presented here. As argued elsewhere, many zones of hummocky moraine in Scotland may simply represent assemblages of types of stagnant ice-sheets at particularly favourable localities, usually determined by the shape of the underlying topography^{13,14}

Finally, the apparently restricted distribution of Loch Lomond glaciers in the eastern Cairngorms contrasts markedly with the wide development of ice caps and outlet glaciers in the Grampians to the south and west^{4, 5, 8, 9, 15}. At present the contrast is difficult to explain

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Distribution of foraminifera in surface waters of a coastal upwelling area

BIOCOENOSSES of planktonic foraminifera are usually accepted to be good indicators of different sorts of oceanic water masses¹, and it is assumed that their ecological requirements have not changed drastically during the Quaternary². Their remains have, therefore, been used for approximately the past 40 yr (ref. 3) to study, qualitatively as well as quantitatively, the palaeoclimatic record⁴ preserved in young, calcareous, deep-sea sediments. Despite this, however, the mechanism of the formation of death assemblages and sediment assemblages is very poorly understood, mainly because the distribution pattern of planktonic foraminiferal biocoenoses and their output of shell material is only poorly known.

Patchiness of faunas, differences between assemblages in different water masses, variability of hydrography over short time intervals and small distances, seasonal fluctuations of fertility and faunal compositions and changing turnover rates usually impede meaningful sampling. These difficulties are even greater in areas such as the continental margin of West Africa, where

Table 1 Varimax factor score matrix

	1	2	3	4
<i>Globigerinoides ruber</i>	0.292	0.001	-0.948	-0.008
<i>Globigerinoides sacculifer</i>	0.938	0.049	0.272	-0.026
<i>Globigerinoides tenellus</i>	0.035	0.021	0.005	0.006
<i>Globigerina bulloides</i>	-0.062	0.946	-0.016	-0.234
<i>Globigerina calida</i>	0.028	0.186	-0.021	0.218
<i>Globigerina falconensis</i>	0.001	0.034	0.008	0.017
<i>Globigerina quinqueloba</i>	-0.085	0.103	-0.103	0.641
<i>Globigerinella aequilatalealis</i>	0.013	0.024	-0.003	-0.010
<i>Globigerinita glutinata</i>	0.075	0.097	-0.006	0.197
<i>Globobulimina dutertrei</i>	0.097	0.153	0.117	0.550
<i>Globobulimina pachyderma</i>	-0.017	0.124	0.017	0.051
<i>Pulleniatina obliquiloculata</i>	-0.011	0.038	-0.008	0.007
<i>Globorotalia inflata</i>	-0.056	0.051	-0.016	0.310
<i>Globorotalia menardi</i>	0.051	-0.046	0.034	0.215

distinct water masses of the central gyres, of the eastern boundary-current system, and of the coastal upwelling zone, interweave in a complicated manner⁵⁻⁷. A preliminary discussion of the planktonic foraminiferal biocoenoses living today in the surface water masses of that region is, however, desirable because of attempts to reconstruct the late Quaternary history of the area using planktonic foraminiferal taphocoenoses in sediments from the continental rise and slope off Spanish Sahara, Mauretania and Senegal⁸.

To minimise the difficulties in adequate sampling and to cover the area as thoroughly as possible, the near-surface water was sampled continuously during Cruise no. 25 of RV Meteor⁸. A pump supplied a set of filters with approximately 1.5 m³ seawater per hour from 4 m below the water surface⁹. Most samples contained 50-200 (maximum 500, >0.12 mm) planktonic foraminifera per cubic meter of filtered seawater. These values are higher than elsewhere in the Atlantic Ocean¹⁰, presumably because of the higher fertility of the surface waters in and around¹¹ the area surveyed.

The planktonic foraminiferal faunas consist of 26 different species⁹. As several of these showed a somewhat patchy and incoherent pattern of distribution, it was decided to reduce the number of variables by applying multivariate techniques¹². I have therefore applied *Q*-mode factor analysis to these samples using the Fortran-IV program CABFAC¹³.

The planktonic, foraminiferal biocoenoses found in the 98 samples of this study resolve into four important factors which together account for more than 90% of the total variance of the studied samples. At first, factor analysis was applied to all 26 species. The calculated factor scores indicate, however, that only 14 species (Table 1) are important contributors to the four factors.

Factor 1 (47% of the variance) is dominated by a group of species confined to the southern and western part of the area studied (Fig. 1a). The most important species within this factor is *Globigerinoides sacculifer*. This and the other significant species of Factor 1 dwell in tropical and subtropical, warm water masses¹. It is thus understandable that this factor shows a zone of minimum loadings along the continental margin of Africa, where a strip of cool water can be traced southwards almost to Cape Vert (Fig. 2).

Factor 2 (18% of the variance) which is dominated by *Globigerina bulloides* (Fig. 1b) has a distribution that is almost exactly opposite to Factor 1. The maximum loadings on this factor occur in the north of the area studied, in the region off Cape Blanc and Cape Barbas. A zone of higher values which can be followed southwards pinches out just north of Cape Vert. A similar distribution of *G. bulloides* in other plankton samples from the region off Cape Blanc has been described¹⁴. This pattern seems to be a rather persistent feature, as it has also been mapped in surface sediments^{2,3}, suggesting that it has existed for several hundred, if not several thousand, years.

The two remaining factors, (factor 3 accounting for 19% and factor 4 for 8% of the variance), also complement each other (Fig. 1c and d). Factor 3 is dominated by *Globigerinoides*

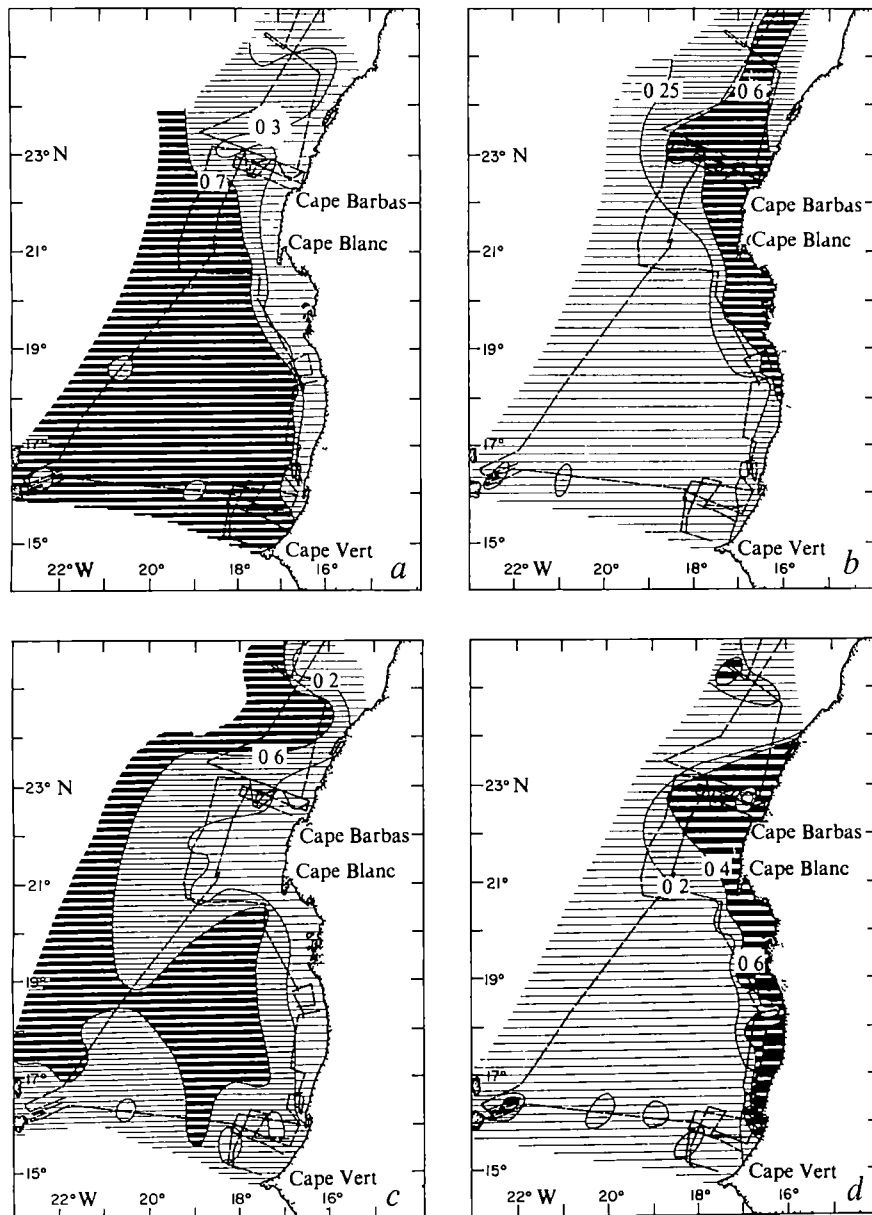


Fig 1 Distribution of planktonic foraminiferal assemblages. Locations of pumping intervals corresponding to surface water plankton samples are marked by heavy lines along the course of the ship RV Meteor cruise no 25, October–December, 1971. Numbers, and associated shading and contours correspond to factor loadings, higher values indicating increasing abundance of respective foraminiferal assemblages: a, Factor 1, tropical warm water; b, factor 2, Canary Current, cool surface water; c, factor 3, tropical warm surface water; d, factor 4, cool upwelled water.

ruber. The regional distribution of this factor shows high loadings far from the coast in the central part of the region studied. That suggests that coastal upwelling has expelled normal surface water from the coastal zone. Thus, species such as *Globigerinoides ruber* which normally dwell in the uppermost layer of the water column are rarely found in the centre of the upwelling zone. If this interpretation is correct, the existence of another factor, caused by foraminiferal assemblages native to the upwelling water masses, could be expected, in fact, factor 4 seems to represent such an assemblage. Its highest loadings are found in the area where the factor 3 loadings are smallest.

Species dominating factor 4 are *Globigerina quinqueloba*, *Globoquadrina dutertrei*, and *Globorotalia inflata*. The highest loadings are found in the inner bight between Cape Vert and Cape Blanc, in samples which are distinctly green in colour because of their very high contents of photosynthetic organisms.

In my opinion, the distribution of this factor, the occurrence of abundant diatoms in all samples⁹ high in this factor and the fact that most of the foraminiferal species are subsurface dwellers¹, clearly indicate that factor 4 is representative of upwelling assemblages in this region. Factor 4 is sharply bounded to the north as well as to the south, and its seaward extension is very limited, the zone of highest loadings is confined to the innermost

few hundred meters. Its extent coincides well with the area of the most intensive coastal upwelling off West Africa¹¹. At first glance, it is puzzling that cool to transitional species, as well as tropical species such as *Globorotalia menardii* are grouped together in this factor (Table 1). In spite of the fact that the temperature of the surface waters are as much as 10°C lower in this region than in the open ocean (Fig 2), *G. menardii* occurs in high absolute and relative abundances in a narrow belt which can be traced along the continental margin from Cape Vert to Cape Barbas, but not further north (Fig 3). This distribution may be caused by a subsurface countercurrent flowing northward along the upper continental slope and outer shelf, presumably carrying *G. menardii* from a region further south where that species occurs most frequently at depth¹⁵. Upwelling of some of the undercurrent to the surface could then produce the anomalous distribution of the species in the studied area. Such an undercurrent is a common feature of upwelling regions^{5,16} and has been identified by deep current measurements⁷ off West Africa.

This pattern of distribution is geologically important, as *G. menardii* has been used widely in subtropical and tropical pelagic and hemipelagic sediments¹⁷ as a biostratigraphic indicator of warm climatic periods of the Pleistocene. Clearly,

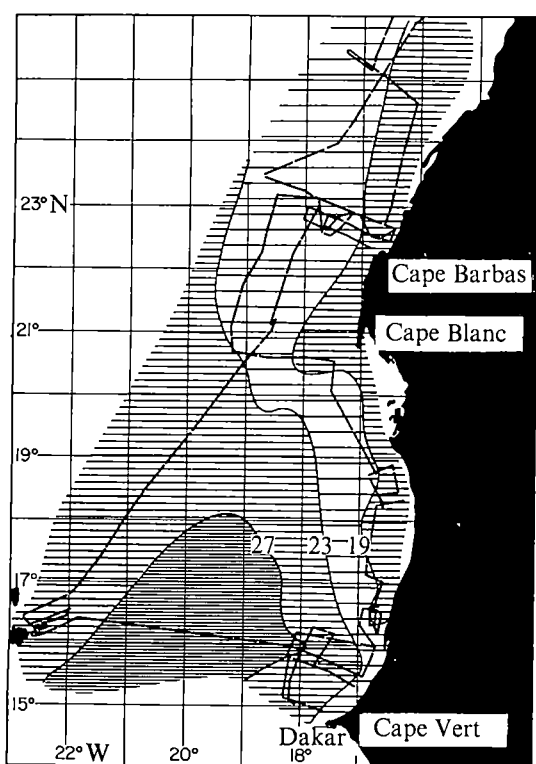


Fig 2 Distribution of sea surface temperatures ($^{\circ}$ C) during late October to early December, 1971 off western Africa. Synoptic interpretation of daytime measurements corresponding to samples used for Fig 1

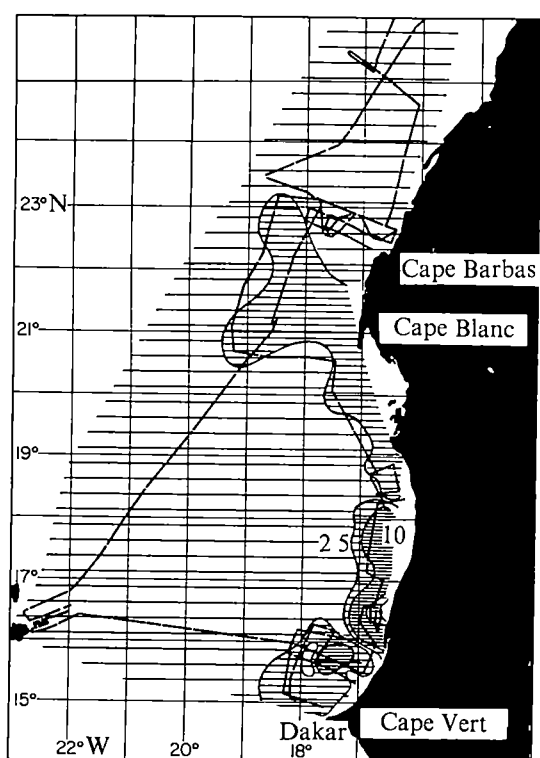


Fig 3 Distribution of *Globorotalia menardii* (%) in surface water plankton samples

the biostratigraphy of deep-sea sediments from upwelling areas must be re-evaluated in light of the possible problems revealed here. Thus, is it possible that intensified trade winds during glacial times trigger off much more intensive upwelling, and could that in turn intensify the subsurface countercurrent? If so, high abundances of *G. menardii* in sediments from likely areas may indicate only intense upwelling, thus presenting exactly the opposite picture from that usually proposed.

Another important point is that most of the species represented by the upwelling factor are highly resistant to dissolution, relative to other species. Although the pattern will be complicated by differing rates of turnover, the presence of the upwelling assemblages in the sediments may give the false impression that there is a heavily dissolved fauna at a depth close to the foraminiferal lysocline¹⁸. Although I suspect that the dissolution of calcium carbonate close to the continental margin is more intense than at comparable depths in the open ocean¹⁹, the data presented here do suggest that faunal indices which have been used to map the depth of the lysocline may be deceptive if the unusual character of the primary shell material is overlooked. Thus, the methods at present used to delineate the lysocline should be applied with caution, at least in upwelling regions, in coastal, and possibly also in equatorial areas²⁰.

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Recent cyclic changes in climate and in abundance of marine life

FLUCTUATIONS in temperature and rainfall are important to agriculture and human populations¹⁻⁶, and there is increasing interest in the possibility of forecasting future oscillations of climate on the basis of their apparent connection with more accurately predictable phenomena such as the sunspot cycle and planetary tidal cycles⁷⁻¹¹. A large part of the solar energy

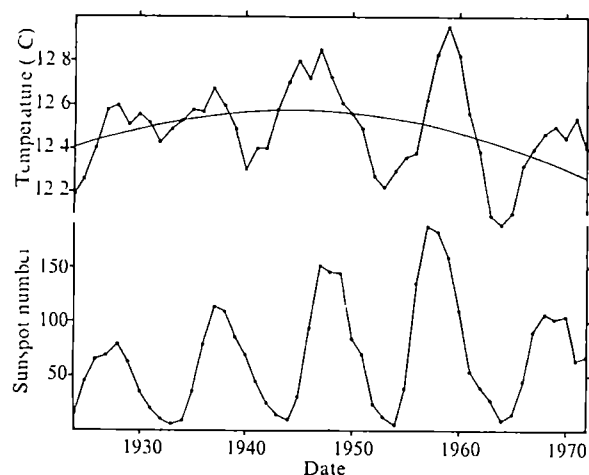


Fig 1 Five-year running averages of sea surface temperature at station E1, compared with mean annual sunspot number (ref 8 and personal communication from H H Lamb). The secular trend of temperature is shown as a smooth curve obtained by fitting a polynomial regression.

budget of the Earth is mediated through the surface of the oceans¹², and it is well established that interaction between the sea, the sea currents and the atmosphere has important meteorological consequences and may also have direct or indirect effects on fisheries¹³⁻¹⁶. During the 1930s several attempts were made to link variations in solar radiation with biological and chemical events in the sea, such as the yield of certain North Atlantic fisheries and the quantity of algal nutrients present¹⁷⁻¹⁹. Tentative predictions made then were clearly ahead of their time, but the longer marine biological records now accumulating show signs of both secular and short period fluctuations which seem to be related in some way to corresponding cyclic fluctuations in sea temperature, and through these, to the solar and other cycles which have been

discussed recently in connection with terrestrial biological cycles^{6,5,20}.

We have analysed sea temperatures taken at International Hydrographic Station E1, situated in the English Channel some 20 miles south of Plymouth, and various biological measurements made in the vicinity. Mid-monthly temperatures and annual means were calculated from the monthly observations^{21,22}, and missing values for the war years have been calculated from mean differences from more continuous observations made inshore in Plymouth Sound^{23,22}, full details of this temperature analysis, including values for 70 m depth, will be given elsewhere. For present purposes the annual means of surface temperature at E1 were subjected to power spectrum (Fourier series) analysis and auto-correlation. Both methods showed the presence of major cycles of the order of 10-11 yr, but longer and shorter harmonics were also present. Filtering off short period variations by means of five-year running averages brings out the 10-11-yr cycle very well and illustrates the close agreement with curves of annual mean sunspot (Zurich) number (Fig 1). Cross-correlation between the 11-yr component of the sunspot cycle and the annual mean temperatures gives an identical phase relationship, a similar phase agreement can be shown between the sunspot cycle and surface temperatures in the North Atlantic²⁴. The absence of any marked phase lag in the more inshore waters perhaps indicates a more direct link through atmospheric events rather than through changes in ocean currents. The short term variations, including the 11-yr cycle can be removed from the temperature records by calculation of a smooth curve (polynomial regression) to fit the annual means. The resulting secular trend is of much smaller amplitude than the shorter cycles but can be compared with the overall trend²⁵ of the sunspot maxima.

The biological observations have been treated in the same way as the temperatures. Calculation of the 5-yr running averages indicates the presence of the same 11-yr cycle in such diverse data as the number of pilchard eggs in the plankton, the catch of demersal fishes and the proportion of barnacle species in the intertidal zone (Fig 2). Removal of the secular trend from the biological data and annual temperatures, and cross correlation of the resulting short period fluctuations, shows significant correlation between temperature and several of the biological variables (Table 1).

Table 1 Biological data

		Cross correlation of temperature and short period trends (<i>r</i>)	Phase lag (yr)	Secular trend linear regression against time (<i>r</i>)
Proportion of southern <i>Chthamalus</i> in the intertidal barnacle zone (1950-74)		0.78	2	-0.79
Catch per hour fishing on Plymouth grounds (RV Sarsia, 1954-72)	Hake	0.50	1	-0.61
	Cod	-0.57	1	(0.23)
Catch per hour fishing by trawlers landing at Newlyn, Cornwall (1948-61)	Hake	(0.24)	2	-0.71
	Cod	-0.69	1	(0.44)
No. of pilchard eggs in a standard plankton haul at station L5 (Eddystone) (1947-74)	Summer	0.52	-3*	(-0.35)
	Autumn	-0.56	1	0.6
No. of non-clupeid postlarval young fish in a standard plankton haul at L5	1924-39	(-0.35)	0-3	-0.80
	1957-74	(-0.26)	3-4	0.87
Inorganic phosphate content of sea at station E1 in winter or early spring ('winter maximum')	1924-38	(0.42)	1	-0.82
	1948-74	(0.28)	1	(0.28)

Values in brackets indicate correlation not significant ($P > 0.05$)

*See text for explanation

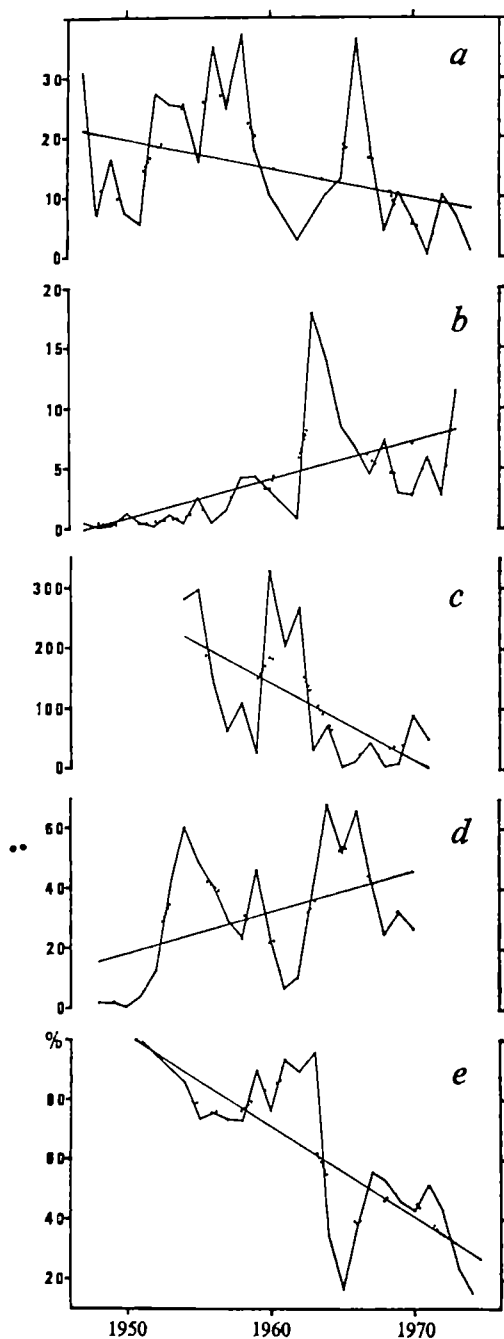


Fig. 2 Biological observations showing short period cycles and longer term trends. *a*, Number of pilchard eggs (thousands) in summer (sum of monthly means for April–July inclusive) at L5, *b*, number of pilchard eggs (thousands) at L5 in autumn (sum of monthly means for September–December inclusive), *c*, catch of hake (as number of fish per 100 h fishing) by RV Sarsia on the Plymouth grounds, *d*, cod landed by Newlyn trawlers (as hundredweights per 100 h fishing) from English Channel grounds, *e*, percentage numbers of *Chthamalus stellatus* (southern species) in total barnacle population on an intertidal traverse at the mouth of the Yealm Estuary near Plymouth —, The annual values, ---, the five year running average. The long term trend is shown as a linear regression

Removal of the short period fluctuations from the biological data reveals the secular trend, and some of the values obtained are also given in the Table, as correlation coefficients of regression against time

In general the warm water species (such as hake, red-mullet, John Dory, megrim, and the barnacle *Chthamalus*) are positively correlated with temperature and the cold water species (such

as cod, haddock and the barnacle *Balanus balanoides*) negatively so. In some the amplitude of the 11-yr cycle is small compared with the secular trend, for example, the proportion of *Chthamalus* in the total population of intertidal barnacles, the catch of hake, the numbers of young fish and the phosphate (algal nutrient) content of the sea before 1939. The secular trend seems to be less important than the shorter period fluctuations in the catch of cod, in the number of pilchard eggs in summer, in the phosphate content of the sea since 1948 and also in sea temperature. Pilchard are classed as warm water fish, the English Channel forming the northern limit of regular commercial exploitation. But the number of pilchard eggs in the autumn is negatively correlated with the 11-yr temperature cycle, and the secular trend is also negatively correlated with the secular temperature trend. This relationship has been discussed elsewhere²⁵ in connection with changes in the fishery and does not invalidate the general inferences drawn here. The summer pilchard eggs show a short period cycle which is in fact in advance of the temperature and sunspot cycle by 3 yr, so the correlation seems to be with the rapidly rising section of the curves rather than with the peaks. In some records, including a few not tabulated here, the long term trend is only vaguely connected with the general direction of the secular trend in temperature and there is little short term cyclic fluctuation, these particular examples, notably the numbers of postlarval young fish, the abundance of certain planktonic invertebrates and the phosphate content of the sea will be discussed fully elsewhere.

Some of the biological trends noted above have previously been found to be related to temperature by calculation of the regressions using the raw data, and a similar one to two year phase lag was shown^{26,27}. But the existence of the 11-yr cycle in these cases explains some of the obvious irregularities that had been found, some data not yet fully analysed suggest that short period cycles are widespread in marine biological and fishery observations, as for example in the catches of other species of fish in the North Sea and English Channel. The longer term trends reported here belong to a family of similar changes which have been found in several parts of the northern hemisphere and have been tentatively linked with the secular trend of sea temperature, these include fluctuations in arctic fishes and invertebrates, phytoplankton and zooplankton of the northern North Atlantic, North Sea fisheries and fish and invertebrates of the English Channel^{13,28-33}.

The peak of the secular temperature cycle was reached in the 1940s or 1950s, and the trend has been in reverse since 1960 or earlier. If the suggested link between the biological trends, the temperature trends, and the solar and planetary cycles is correct, and if the predictions for the latter phenomena are correct^{7,11}, then the temperature trend should continue in reverse until 1990 or later, each successive 11-yr cycle bringing about a temporary re-reversal of lesser and lesser amplitude. On this basis it seems possible that we will see in the English Channel (and in other seas) further increases in the abundance of cold water species and decreases of warm water forms. It was remarked, concerning the rising phase of the secular trend, that the change in fauna had been detrimental to local English Channel fisheries³¹ (though beneficial in the Arctic²⁸), as many of the warm water species were of lesser commercial value than the cold water species they replaced. Some improvement to western English Channel fisheries has already taken place during the past 10 yr while the trend was in reverse, notably a return of cod and haddock and increases in mackerel shoals in certain areas, though there is a local belief that these effects are a benefit of the exclusive 12 mile coastal fishing zone recently claimed. In these days of closure of distant water northern fishing grounds and increasing fuel costs, a small improvement in near water fishing in the English Channel and adjacent seas may prove to be more beneficial than anticipated earlier.

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Physical basis of dielectric loss

THE frequency dependence of the dielectric loss of all kinds of dielectric solids follows a 'universal law' ω^{-n} , with $n < 1$ regardless of their physical and chemical nature. I show that, rather than being the result of superposition of Debye-like loss peaks, this is the manifestation of a universal mechanism for which the ratio of the energy lost per cycle to the energy stored per cycle is independent of frequency. A physical model is proposed which should apply generally to most dielectric materials and which has the required property.

The traditional approach to the interpretation of the frequency dependence of the dielectric loss is based on the Debye polarisation mechanism for which the complex dielectric susceptibility $\chi(\omega)$ is given by

$$\chi(\omega) = \chi'(\omega) - i\chi''(\omega) = [\epsilon(\omega) - \epsilon_\infty]/\epsilon_0 = (1 + i\omega\tau)^{-1} \quad (1)$$

where $\epsilon(\omega)$ is the complex dielectric permittivity at a frequency ω , ϵ_∞ is the limiting high-frequency value of ϵ , ϵ_0 is the permittivity of free space and τ is the dielectric relaxation time. This produces the familiar Debye loss peak at $\omega\tau = 1$ which is symmetric on a $\log \omega$ plot and has a half-width $\lambda = 1/144$ decades.

In general the loss does not obey equation (1) except in some liquid dielectrics and departs seriously from it in most solids. The commonly accepted interpretation of this invokes

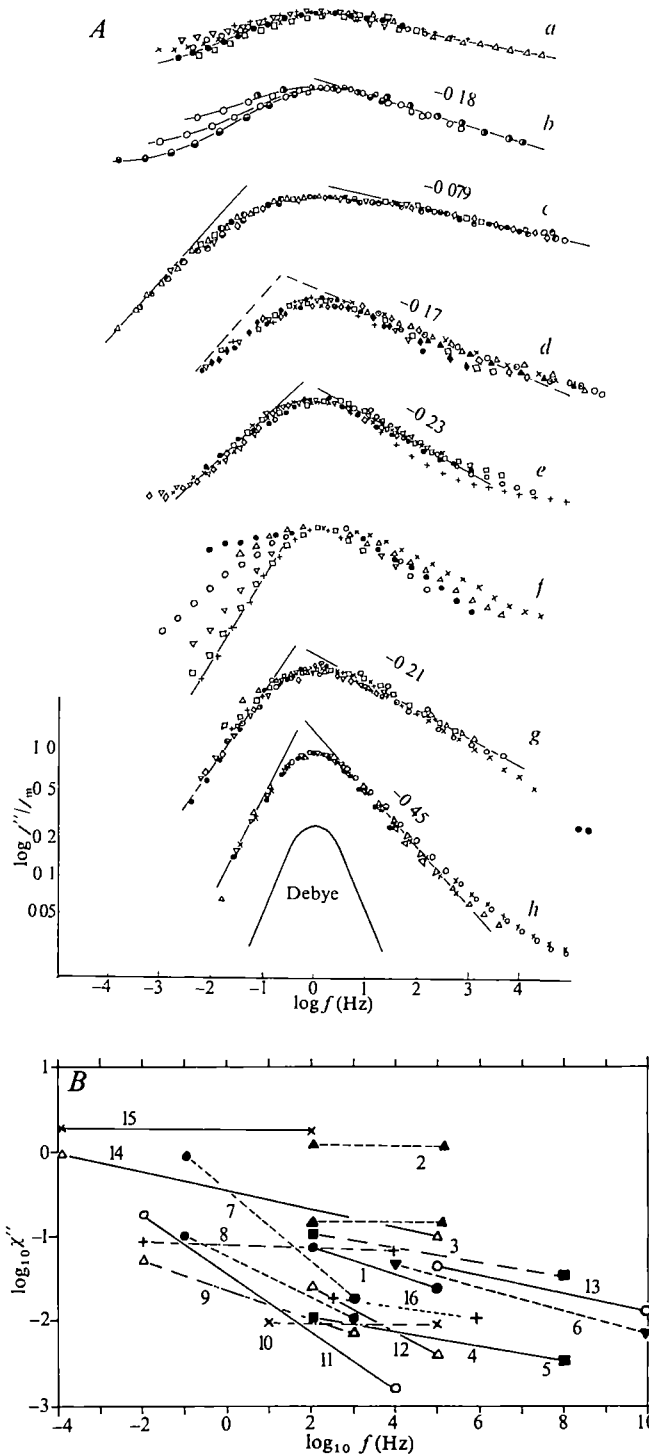


Fig 1 A, A compilation of dielectric loss data for a range of polymeric materials². The points marked with different symbols correspond to data taken at different temperatures and transposed laterally to make the loss peaks coincide. The vertical scale is $\log \chi''$ and the consecutive graphs are displaced vertically for clarity. The numbers indicate slopes of the straight line portions of the graphs. a, Low temperature data for polychloroprene, b, PCTFE 12% crystalline, c, polyethylene terephthalate, d, polymethyl methacrylate, e, high temperature data for polychloroprene, f, polyethyl methacrylate, g, polydian carbonate, h, polyvinyl acetate. The shape of a pure Debye peak is also included for comparison. B, The corresponding compilation of loss data for a range of dielectric materials, including some amorphous inorganic solids, taken from ref 4. Number code 1, compensated single crystal silicon in the impurity hopping range, 2, $\text{Sb}_2\text{As}_2\text{S}_3$, 3, As_2Se_3 , 4, As_2S_3 , 5, Se, 6, As_2Se_3 , 7, SiO_2 range of temperature, 8, SiO_2 , 9, Si_3N_4 , 10, Al_2O_3 , 11, Trinitrofluorenone, 12, solid polymerised CS_2 , 13, Cu-Phthalocyanine, 14, Polyvinyl chloride, dioctyl phthalate, 15, Stearic acid, 16, Supercooled aqueous solution of LiCl.

a distribution of relaxation times, $g(\tau)$ such that the observed loss function is represented formally by the integral transform

$$\chi''(\omega) = \int_0^\infty g(\tau) \frac{\omega\tau}{1 + \omega^2\tau^2} d\tau \quad (2)$$

Examining the experimental evidence I conclude that equation (2) has little meaning but the observed frequency dependence of loss has a simple physical significance¹ and I propose a model which should be generally applicable. When the loss of many polymeric materials is plotted as $\log \chi''$ against $\log \omega$ with temperature as parameter, one obtains families of curves which may be 'normalised'² by shifting along the frequency axis (see Fig 1) which gives a compilation of data for several materials. The loss may be represented by the empirical relation

$$1/\chi'' = (\omega/\omega_2)^{-m} + (\omega/\omega_1)^{(1-n)} \quad (3)$$

where $1/\omega_1$ and $1/\omega_2$ are thermally activated parameters with well defined activation energies. The exponents m and n are both smaller than one, and m is always greater than $(1-n)$ by a factor between 2 and 6 depending on the particular polymer and on temperature, resulting in a marked asymmetry of the peaks. Both m and $(1-n)$ tend to decrease with decreasing temperature resulting in very broad peaks having half-widths of 6 decades or more. The low-temperature β peaks are much broader and less symmetrical than the higher temperature α peaks³.

In addition to polymers, the dielectric loss in a wide range of inorganic materials in which the conduction is at least to some extent caused by hopping charge carriers¹ follows a law corresponding to the second term in equation (3) over a wide range of frequencies (ref 4, Fig 1B). But in these materials there is seldom a well defined loss peak at lower frequencies, instead the d.c. conductivity σ_0 dominates by contributing a term $\sigma_0/\epsilon_0\omega$. So I suggest that the dielectric loss in a very wide range of materials follows over a wide range of frequencies a 'universal law of loss'

$$\chi''(\omega) = B(T)\omega^{(n(T)-1)} \quad (4)$$

where $B(T)$ is a temperature dependent parameter.

The 'universal law' (4) cannot be plausibly explained in terms of $g(\tau)$ for many reasons, here I mention two of them. The physical difficulty of explaining the required range of τ values spanning not less than 8 to 10 decades, and the difficulty of understanding how the same power law (4) should result, albeit with different values of n , for most known solid dielectric materials, be they dipolar, electronic, ionic, organic, inorganic, polymeric, crystalline, amorphous, and so on.

Although the examples of Fig 1 relate to selected simple cases which approximate to relations (3) or (4), I have shown (unpublished) that in many other cases where the loss exhibits a more complex behaviour it is possible to resolve the frequency dependence of both $\chi'(\omega)$ and $\chi''(\omega)$ into a superposition of two, or at most three, overlapping mechanisms of the type (3). I suggest that equation (3) is a more 'natural' function in which to 'expand' the experimentally observed loss data than the Debye function, which requires the assumption of a rather arbitrary distribution function $g(\tau)$. Using the universally applicable Kramers-Kronig relations, the empirically observed law (4) for $\chi''(\omega)$ implies the same frequency dependence for the real part $\chi'(\omega)$, so that the ratio χ''/χ' is independent of frequency¹. This should be contrasted with the Debye behaviour where this ratio is equal to $\omega\tau$. The consequence of this is that the ratio

$$\frac{W_l}{W_s} = \frac{\text{Energy lost per cycle}}{\text{Energy stored per cycle}} = \cotan\left(\frac{n\pi}{2}\right) = \text{constant} \quad (5)$$

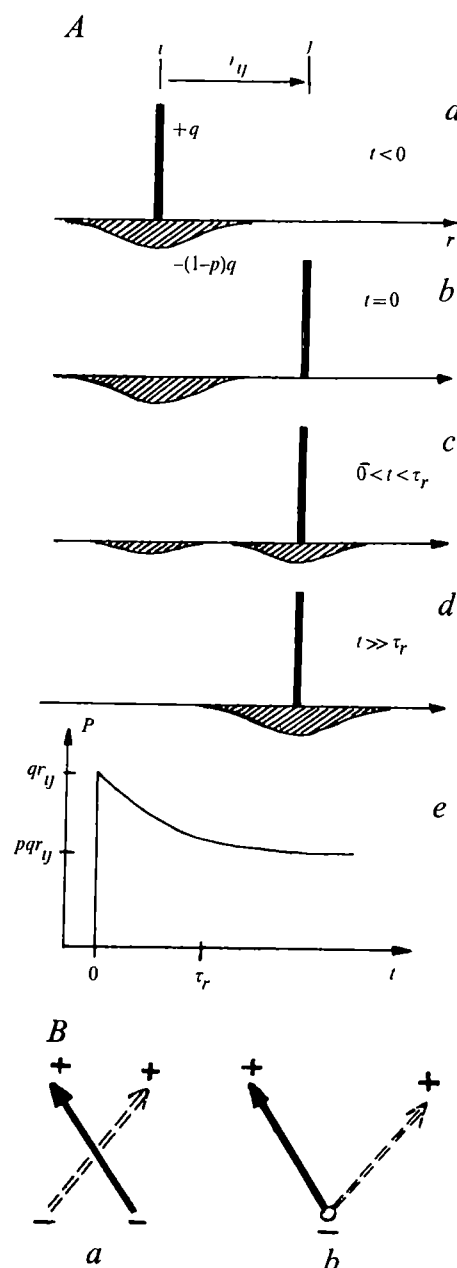


Fig 2 A, The discontinuous jump of a charge q from a localised site i to a site j followed by a slower transfer of the screening charge. Diagrams $a-d$ show consecutive stages, e shows the time dependence of the polarisation P resulting from the transition from i to j . B, The contrast between a 'free' dipole (a) and a 'pinned' dipole (b). A change of the orientation of the former does not bring about any change of the distribution of space charge, while a similar change for the latter does.

with respect to frequency I suggest that this physically very simple criterion is the universally applicable common feature of all dielectric materials obeying equation (4). Conversely, if relation (5) is applicable, then the frequency law (4) is the only possible frequency dependence of loss.

What physical mechanism leads directly to the criterion (5)? I begin with materials in which there exist significant densities of localised electronic or ionic carriers capable of random hopping between spatially fixed centres. These materials often possess a significant d.c. conductivity and they are in many cases structurally strongly disordered. The presence of hopping conduction is an essential element of my present argument, free carrier conduction in conduction and valence bands lies outside its scope.

Consider now the hopping equivalent of a Debye dipole a charge $+q$ which may hop between two localised sites i and j separated by a distance r_{ij} . The natural frequency of hopping between the two sites is $1/\tau_D$, where τ_D is a thermally activated Debye relaxation time. The loss resulting from this mechanism is given by equation (1) if no further reservations are made.

But our dielectric material contains a certain density of these localised charges, some of which may be able to hop over many consecutive sites ultimately giving rise to a d.c. conductivity σ_0 , while others may be restricted to shorter ranges, with the limiting case of pairs of hopping sites. A charge $+q$ localised on a site i imposes a Coulomb-like potential on the surrounding medium and this tends to repel like charges and to attract any oppositely charged particles that may be present in the neighbourhood, thus bringing about a partial screening of the charge in question. In the case of a gas of free carriers we obtain the screened Coulomb potential which implies that the effective charge decreases rapidly to zero outside a sphere of a few Debye lengths in diameter.

In a system consisting of localised charges the screening would not be as complete as that due to free charges, since localised charges are not quite free to assume the exact local density demanded by the local value of the potential. Nevertheless, we may postulate that the effective value of the charge q on site i is reduced to some smaller value pq , where $p < 1$.

If now the charge in question makes a rapid hopping transition to a neighbouring site j through a distance r_{ij} , the screening charge will initially be left behind on site i , so that the initial change of polarisation is qr_{ij} and this only gradually decreases to pqr_{ij} as the screening readjusts itself to the new position of the charge at j . I assume that this adjustment takes a time which may be loosely characterised by a relaxation time τ_r , without implying necessarily any particular form of time-dependence of this process. Assuming that the charge remains at site j for a time longer than τ_r (that $\tau_r < \tau_D$), the sequence of events involved in a hopping transition may be visualised with reference to Fig. 2A. The reduction of the resulting polarisation from qr_{ij} to pqr_{ij} corresponds to the reduction of the potential energy of the system discussed before¹ and the energy loss involved is dissipated through the phonon system.

The opposite case where $\tau_D \ll \tau_r$ is more likely to occur in nature and presents a qualitatively different picture, although the end result is the same. Since the screening space charge cannot follow the individual transitions, the screening charge adjusts itself to the time-averaged occupancies f_i and f_j of the two sites. The application of an external field E enhances the down-field rate and reduces the up-field rate resulting in a net change of occupancies by $\pm f'$ with respect to equilibrium. Each individual 'net' transition may be considered as the transfer of a 'bare' unscreened charge q through the distance r_{ij} , but the subsequent readjustment of the screening charge gives rise to a final polarisation $P = pqr_{ij}f'$. Since all the net transitions take place in the field E , the energy derived from the field is $Eqf'r_{ij}$ but the energy stored in the system after relaxation is only

$$W_s = Eqpr_{ij}f' \quad (6)$$

and the energy lost is the difference between these two

$$W_1 = Eq(1-p)r_{ij}f' \quad (7)$$

I conclude that in either case, $\tau_r < \tau_D$ or $\tau_D < \tau_r$, the setting up of a given state of polarisation P in the system of hopping charges must give rise to a loss of energy which is proportional to the stored energy and which does not depend on the rate at which this state of polarisation has been established.

The energy loss W_1 arising from the proposed screening mechanism is additional to any loss that may arise in an alternating electric field from the normal Debye delay τ_D in the response of hopping charges which gives rise to a loss given

by equation (1) and which may be considered negligible a decade or two below the frequency $1/\tau_D$. I am here concerned with losses extending down to much lower frequencies. The conditions which therefore have to be satisfied in a dielectric system for the universal criterion (5) to apply are (1) discontinuous hopping of charges between localised positions, and (2) the presence of a screening charge adjusting slowly to the rapid hopping.

I have already stressed the point that the similarity of the functional dependence $\chi''(\omega)$ in hopping charge and in dipolar systems above the loss peak makes it plausible to look for essentially similar physical mechanisms of loss in both. How can this similarity arise despite many apparent differences? Dipoles in solids are not free to rotate as the idealised Debye model would require, but are constrained to assume discrete orientations in space with respect to the nearest neighbours, regardless of whether the material is crystalline or amorphous, since there is little difference between them in the short range order. Transitions between these discrete orientations occur abruptly in a manner completely analogous to the translational hopping of localised charge carriers, as required by condition (1) of my model.

An ideal dipolar system cannot give rise to screening, since no transfer of charge can occur. But all real molecular dipoles have finite length on an atomic scale and invariably one of their poles is more rigidly fixed in the 'lattice' than the other, as for example in the case of a polar side group on a rigid carbon chain in a polymer. I therefore introduce the concept of a 'pinned dipole', the response of which is not purely orientational but contains also an inseparable element of charge translation (Fig. 2B). Thus, pinned dipoles screen fields just as hopping charges do, but possibly less effectively.

Experimental results show clearly that the dielectric loss in most solids shows a simple power-law frequency dependence and this implies, as a consequence of Kramers-Kronig relationships, a frequency-independent ratio of energy lost per cycle to energy stored per cycle. I have suggested a model which satisfies this energy criterion and which is equally applicable to materials in which polarisation results from hopping of charge carriers and to those in which it results from dipolar molecular processes.

The proposed mechanism is much more plausible than the accepted interpretation in terms of a distribution of relaxation times. The physics of loss processes at and below the loss peak frequency will be the subject of a separate treatment.

The present work attempts to treat the dynamics of dielectric polarisation as a many-body interaction, by analogy with Calogero-Mosotti's static approach. The particular physical model proposed is not necessarily unique but the energy criterion (5) itself is well established by experimental facts.

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Electricity from photosensitisation of titanium

FUJISHIMA and Honda¹ have described a novel photochemical cell with an open circuit e.m.f. of up to 0.5 V which they indicated had a capability for water photolysis. The anode consisted of a TiO₂ n-type single crystal which was illuminated by light of wavelength shorter than 415 nm. This corresponds to the 3.0 eV band gap of TiO₂ (refs 2-4).

• It has occurred to us that a photosensitive electrode for a photochemical cell might be formed equally well by oxidising a titanium electrode, and tests show that this seems to be the case. A very convenient way of making such a photosensitive surface is to pass a current between the Ti electrode and a platinum electrode both of which are immersed in an aqueous solution of 5 N KOH. The direction of current is such that the titanium electrode is the anode, and it consequently acquires an increasingly dark blue appearance as electrolysis continues.

In our experiment the oxidised titanium electrode (anode) and a platinum electrode (cathode) were mounted in the arms of a eudiometer to facilitate the measurement of gas uptake and evolution, with 5 N KOH as electrolyte. When the anode (fully immersed in electrolyte) is illuminated by a 100 W high pressure mercury lamp, and the cathode is partially exposed to air, oxygen is evolved on the illuminated face of the anode and current of the order of 1 mA cm⁻² flows. The open circuit voltage of the cell is approximately 1 V. The amount of exposed cathode area has no effect on these characteristics provided the length of the cathode-electrolyte-air interface is constant. But if the cathode is fully immersed in the electrolyte or if the air above it is replaced by nitrogen, the evolution of oxygen at the anode ceases and the current is greatly reduced.

So it seems, that oxygen is, under these conditions, adsorbed at the cathode-electrolyte interface, our measurements indicate that the volume of oxygen released at the illuminated anode surface is almost as much as the amount adsorbed. Gas evolution under these circumstances has not been observed at the cathode, but addition of an external e.m.f. of ~0.3 V results in the release of hydrogen at the cathode. This is consistent with the theoretical balance potential of 1.23 V. If a reduced TiO₂ single crystal is substituted for the titanium anode, the cell characteristics already noted remain essentially the same. We note that Boddy⁵ has also observed anodic evolution of oxygen at a rutile crystal-electrolyte interface.

The surface of titanium exposed to air develops a yellowish colour⁶ indicative of TiO. When a piece of untreated titanium is substituted for the electrolytically-treated titanium anode in the cell and connected to the cathode, a small current flows under illumination. Eventually it develops the same characteristics as the electrolytically-treated anode.

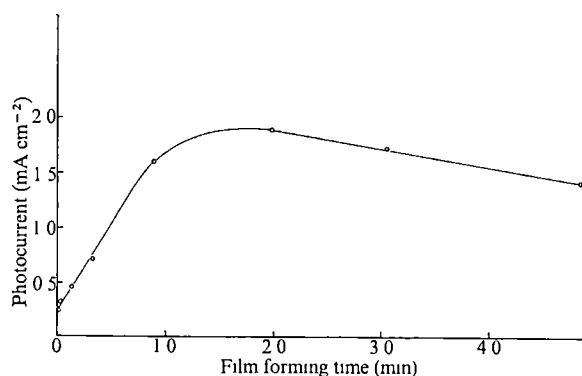


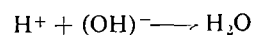
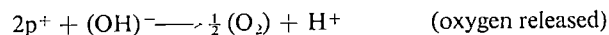
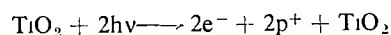
Fig. 1 Short circuit output photocurrent of oxidised titanium as a function of electrolytic forming time at 8 mA cm⁻².

The anode, which was formed electrolytically under constant current, was placed in the cell at intervals to determine the short circuit photocurrent capability (Fig. 1). There seems to be an optimum forming time.

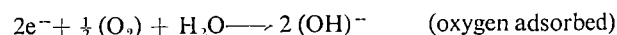
We have attempted to measure the corresponding weight increase of the titanium electrode and can establish an upper limit for the number of oxygen atoms as 10¹⁸ cm⁻².

If the concentration of KOH is reduced from 5 N to 1 N, production of oxygen is greatly reduced while the photocurrent remains substantially the same.

This suggests that more than one mechanism may be involved in our observations. One of these may be the following:

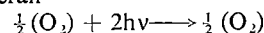


at the anode, and



at the cathode

Overall



Fujishima and Honda¹ have observed that the addition of more reducible species to the cathode compartment such as dissolved oxygen or Fe³⁺ serves to increase greatly the current flow. This leads us to believe that a scheme similar to that described above may play a part in their cell.

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Ionic conductivity in some bivalent metal sulphides

INVESTIGATIONS of the ionic conductivity of solids have been concentrated mainly on materials characterised by a purely ionic conductivity, such as AgI, RbAg₄I₅, and β-alumina, which have practical applications in the production of solid-state high energy-density batteries and other electrochemical devices¹. In such cases a non-zero electronic contribution prevents the material from acting as a solid electrolyte. Materials with mixed ionic-electronic conductivity can be used as electrode materials in electrochemical cells.

A few materials (mainly oxides and sulphides) characterised by this type of conductivity have been studied. Investigations have been carried out on monovalent metal sulphides. Partial ionic conduction, dependent on stoichiometry, was detected by Hebb² in Ag₂S and by Hirahara³ in Cu₂S. Bivalent sulphides, however, have not been covered in the literature. But among these compounds are materials of considerable technological interest, such as ZnS. The existence of mixed ionic-electronic conductivity could be important to explanations of their behaviour as electronic devices.

By measuring the e m f of suitable galvanic information cells¹, the ionic and electronic contributions to the electrical conductivity of some bivalent sulphides—such as the 2B group sulphides ZnS, CdS, HgS, PbS and CuS—have been determined

We prepared samples comprising reagent grade chemicals. They were prepared from different raw materials and by different methods: precipitation and idrothermal synthesis. Impurity levels were determined spectrographically and were found to be 50–100 p.p.m. for ZnS and CdS (major impurities Cu, Fe, Mg) and 50–150 p.p.m. for the other sulphides.

For each sulphide, the formation cell Me/MeS/S was established, Me consisted of a metal foil (a liquid Hg metal drop for HgS), MeS was a sulphide pellet (prepared by pressing the powder at 5 ton cm⁻²) which worked as the electrolyte, and the sulphur electrode consisted of rhombic sulphur, melted with 20% graphite powder and poured on the sulphide pellet.

The e m f was measured at 25° C by means of a 10¹⁴ ohm input impedance Keithley 630 potentiometric electrometer.

Table 1 Measured, E , and calculated, E^0 , e m f of some galvanic cells

Cell	Crystallographic phase of MeS	E (V)	E^0 (V)
Zn/ZnS/S	Cubic	0.94 ± 0.01	0.98
	Hexagonal	0.83 ± 0.01	0.98
Cd/CdS/S	Cubic	0.69 ± 0.01	0.75
	Hexagonal	0.62 ± 0.01	0.75
Hg/HgS/S	Cubic	0	0.23
Cu/CuS/S	Hexagonal	0	0.25
Pb/PbS/S	Cubic	0	0.50

The average readings, E , are reported in Table 1 where they are compared with the E^0 values calculated from the standard ΔG^0 value of formation of the sulphide under examination⁵.

The reproducibility of E was found to be within ± 10 mV, using different samples with different impurity levels, prepared using different techniques.

Accepting certain assumptions⁴, the e m f of the cell can be expressed as a function of the sole transport number, t_e , of the electrons in the electrolyte

$$E = (1 - t_e) E^0$$

and E^0 is in fact the value that E would achieve if the metal sulphide were to behave as a purely ionic conductor ($t_e = 0$).

The ionic transference number, t_i , of the sulphide may be obtained easily from the simple relationship

$$t_i = (1 - t_e) = E/E^0$$

where t_i represents the sum of all possible ionic contributions. In the case of the compounds considered here, however, t_i practically coincides with the transference number of the cation. The results are shown in Table 2, together with the

Table 2 Ionic transference number, t_i , and total conductivity, σ of some metal sulphides

Sample	Crystallographic phase	σ (Ω cm) ⁻¹	t_i
ZnS	Cubic	3×10^{-7}	0.95 ± 0.01
	Hexagonal	10^{-6}	0.84 ± 0.01
CdS	Cubic	5×10^{-7}	0.92 ± 0.015
	Hexagonal	10^{-4}	0.83 ± 0.015
HgS	Cubic	5×10^{-1}	0
CuS	Hexagonal	5×10^{-1}	0
PbS	Cubic	10^{-2}	0

crystallographic phase and the total a.c. conductivity of the samples examined.

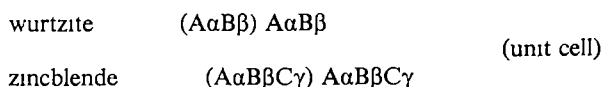
We found that zinc and cadmium sulphides are characterised by mixed conductivity, which has a significant ionic contribution. These sulphides have a very low total electrical conductivity which is strongly dependent on the frequency of the a.c. signal used, in the range 10²–10⁴ Hz.

On the other hand, in Cu, Hg and Pb sulphides, the contribution from the ionic conduction is practically zero. This group of sulphides is characterised by a higher total conductivity with negligible frequency effect.

Before we discuss these experimental results, we should first remark that, besides other factors, structural features have a decisive role in enhancing ionic conduction in solids.

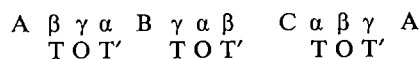
Availability of empty sites in the structure, where ions could jump from normally occupied sites, can be thought of as the essential condition before ions can significantly contribute to charge transport. That alone, however, does not seem sufficient in cases in which the existence of very narrow passage ways between occupied and empty sites should result in extremely high activation energies associated with the ion movement; other mechanisms would have to be operative. That seems to be the case with zinc and cadmium sulphides, as can be recognised by examining the ZnS structure in detail. This sulphide crystallises⁶ at low temperatures in the cubic zincblende form and at high temperatures in the hexagonal wurtzite form, with a transition temperature of $1,020 \pm 5^\circ$ C. A rhombohedral form and different polytypes also exist. The structures of zincblende and wurtzite are strictly correlated.

In both structures zinc and sulphur atoms are surrounded by four atoms of the other element at the apices of a regular tetrahedron. The second coordination sphere is very similar in the two structures, there is, however, some difference in the rhombohedral form. By considering the two structures along the close-packing direction (in this case zincblende is assumed to have a hexagonal cell) they can be schematised as



where A, B, C and α , β , γ indicate the close-packing positions occupied by S and Zn, respectively.

In fact, three cationic layers—one with octahedral (O) and two with tetrahedral (T) sites—are available between the two sulphur layers. Only one of them is usually occupied (T'), as in the case of zincblende



If the cationic and anionic sublattices are equivalent, this is also valid for sulphur atoms, but their contribution to ionic conduction can be questioned.

In an ideal structure, consideration of the ionic radii (Zn, 0.74 Å, S, 1.84 Å) shows that tetrahedral sites are very narrow and an atom is embedded at the centre of the tetrahedron, high activation energies can be expected for the ionic conductivity. The Zn–S bond is only partially ionic, thus atomic radii are nearer to tetrahedral covalent radii⁷ (Zn 1.31 Å, S 1.04 Å), but that does not vastly increase the freedom of movement.

The ionic conductivities of ZnS and isomorphous CdS at room temperature are orders of magnitude higher than those of alkali or alkali-earth halides at the same temperature. The presence of structural defects apart from empty sites and point defects, which enhance ion transport, can therefore be guessed at.

This seems to be particularly so in the sulphides discussed here, as they could contain domains of a second phase. As mentioned before, both phases contain empty sites, which

could be made accessible by stacking faults at the domain boundaries, so they could work as additional pathways for mobile ions. In fact, broken tetrahedral bonds are present to a large degree, because of the formation of stacking faults⁸. The not so different values obtained for the ionic transference numbers in zincblende and wurtzite phases (Table 2), are in agreement with the assumed model and can be justified because of the accuracy of measurement and because of variations of the total conductivity of samples.

On the other hand, in HgS we have not found any ionic contribution to conductivity. This compound, however, also exhibits two phases: cubic metacinnabar (isomorphous with zincblende) and trigonal cinnabar. The latter can be considered as a strongly distorted NaCl-type phase, without empty sites in the lattice. Thus, the absence of ionic conductivity in cubic HgS seems to emphasise the role played by two phases, both with empty sites and with slight mutual differences, in the contribution of ions to electrical conductivity, as we have observed in ZnS and CdS.

As for the other bivalent sulphides examined: PbS and CuS, they have only a crystallographic form, NaCl-type and hexagonal, respectively. Bearing in mind our previous considerations, their purely electronic conductivity is understandable. The enhanced electronic conductivity (with respect to ZnS and CdS) of those sulphides also reflects the structural differences, but we will not consider that here.

The presence of a mixed ionic-electronic conductivity in Zn and Cd sulphides holds a high technological interest in that such compounds are used in a number of electronic applications (electro and cathodo luminescent materials, photocells, and so on). As a matter of fact, the lifetime of these kinds of devices may be affected by ionic mobility, which could be considered as responsible for their ageing. Phenomena of this type are actually characteristic of the devices considered, and their behaviour should be reconsidered in the light of our results.

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Novel phosphosilicate

Few studies of materials containing both phosphate and silicate tetrahedra have been reported. This may seem surprising as large numbers of natural or synthetic aluminosilicates and aluminophosphates are known^{1,2}. But the substitution in minerals of P(V) for Si(IV) in silicate structures, or of Si(IV) for P(V) in phosphate structures is not common. Among the minerals described to have any significant degree of P(V)-Si(IV) partial substitution are visseite³, nagatelite⁴ and wilkeite⁵, an apatite with partial $\text{SiO}_4 + \text{SO}_4$ substitution for PO_4 . Some synthetic phosphosilicates, chiefly apatites, are described in refs 6 and 7. A very few phosphosilicates where Si and P may be crystallographically independent are also known, and the structures of silicocarnotite⁸ [$\text{Ca}_5(\text{PO}_4)_2\text{SiO}_4$] and lomonoso-

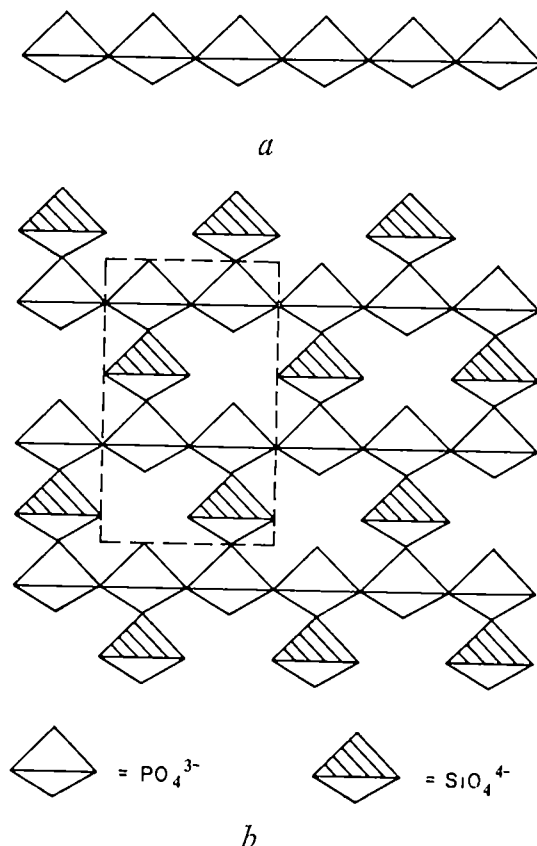


Fig 1 One possible schematic representation of tetrahedra in the $\text{P}_2\text{SiO}_8^{2-}$ framework which may occur in $\text{VO}(\text{P}_2\text{SiO}_8)$. *a*, Shows a schematic metaphosphate $(\text{PO}_3)_\infty$ linear chain, *b*, shows how these chains might be linked by SiO_4 tetrahedra (the dotted line encloses a $\text{P}_4\text{Si}_2\text{O}_{16}^{4-}$ unit). No particular conformation of the 'layer' thus formed is implied. The 'layer' may also be derived from the one-dimensional pentaphosphate ribbon structure found²³ in $\text{NdP}_5\text{O}_{14}$, if the phosphorus in the cross-linking tetrahedra in the ribbons is replaced by silicon, and the ribbons are then linked together by silicate tetrahedra.

vite⁹ [$\text{Na}_5\text{Ti}_2(\text{Si}_2\text{O}_7)(\text{PO}_4)\text{O}_3$] have recently been described. Natural phosphorus is mostly found in association with calcium in apatite minerals^{1,2}.

Using hydrothermal techniques, Barrer and Marshall² were unable to prepare visseite or any other material with substitution between Si and P, although many aluminosilicates and aluminophosphates were obtained, phosphorus substitution in several silicate zeolite frameworks was achieved by Flanigen and Grose¹⁰ using a hydrothermal gel crystallisation technique. Otherwise, except for the chemical analysis of minerals such as visseite and wilkeite, few systematic studies of phosphosilicates have been made, and this limited attention is no doubt the result of their infrequent occurrence in nature. The reason for this lack of Si(IV)-P(V) substitution is not entirely clear, but may reflect the lack of appropriate geological preparative conditions, which allowed the formation of relatively more stable species such as the apatites, rather than any fundamental instability.

Many possible formulae for phosphosilicates may be derived, however, and new crystal structures with perhaps interesting properties may be envisaged. We report here the preparation and some properties of oxovanadium (IV) diphosphatomonosilicate, $(\text{VO})^{2+}(\text{P}_2\text{SiO}_8)$. The stoichiometry of this material may indicate a novel arrangement of condensed tetrahedra, and the ease of preparation indicates that phosphosilicates may indeed be a potentially extensive class of materials.

Small (up to 1 mm) blue-green crystals of $\text{VO}(\text{P}_2\text{SiO}_8)$ were prepared by vapour transport in a quartz tube using iodine as the transporting agent and $\text{VO}(\text{PO}_3)_2$ as the starting material.

(VO(PO₃)₂) was prepared by reduction of V₂O₅ by hot phosphoric acid. Its properties, and those of V³⁺(PO₃)₃, prepared in a similar fashion, will be described elsewhere.) Transport was obtained with the hot zone at 1,100 °C. X-ray emission analysis of a single crystal indicated the probable stoichiometry and this was confirmed by preparation of polycrystalline material by solid state reaction. 1:1 molar pre-dried VO(PO₃)₂ and SiO₂ were heated for 2 d at 1,040 °C in a sealed platinum tube. The X-ray powder pattern of the polycrystalline material was identical to that of a crushed single crystal, and quite different from those of the starting materials. The polycrystalline preparation was analysed for total vanadium, phosphorus and silicon by the Schwarzkopf Microanalytical Laboratory, Woodside, New York 11377. The calculated proportions by weight were: V, 17.9%; P, 21.7%; Si, 9.9%. The proportions found were: V, 17.9%; P, 22.0%; Si, 9.5%. The incorporation of silicon into crystals grown on the tube walls from the vapour is not uncommon in experiments carried out in quartz tubes. For example, the preparations of Ba=Nb₁₄SiO₄₇ (ref. 11) and Yb₃(SiO₄)₂Cl (ref. 12) have been recently described. Precession photographs of a single crystal (Mo K α radiation) of VO(P₂SiO₈) indicated the tetragonal space group P4/n.c.c. (No. 130) with cell dimensions $a_0 = 8.697$ (8) Å and $c_0 = 8.119$ (8) Å. Conoscopic viewing of the optic figure confirmed the crystal to be uniaxial. The measured density (2.9 ± 0.1 g cm⁻³) indicates four molecules per unit cell.

In common with VO(PO₃)₂ and V(PO₃)₃, VO(P₂SiO₈) may be heated to at least 1,000 °C in air without oxidation or decomposition, and is insoluble in most non-oxidising solvents. These unusual characteristics for reduced vanadium compounds entailed the characterisation of the vanadium species by magnetic and spectral methods rather than by chemical analysis of the oxidation state. The magnetic susceptibility was measured on a polycrystalline sample between 1.5 K and room temperature using a pendulum magnetometer. Curie-Weiss behaviour was observed between 300 K and 10 K with $\mu_{\text{eff}} = 1.70\mu_B$, which is characteristic¹³ of VO²⁺ compounds (3d¹). The paramagnetic Curie temperature is 0 ± 1 K but antiferromagnetic ordering is observed below 2.5 K. Magnetic ordering is not frequently observed in d¹ compounds ($S = \frac{1}{2}$), but β -VOSO₄ is reported¹⁴ to be antiferromagnetic below 25 K and α -VOSO₄ ferrimagnetic below 4 K. Also, VF₄ is a canted antiferromagnet below 28 K (ref. 15). The diffuse transmission electronic spectrum of polycrystalline VO(P₂SiO₈) shows three bands centred at 12,500 cm⁻¹, 15,250 cm⁻¹ and 27,000 cm⁻¹, characteristic of V⁴⁺ species containing a distorted VO₆⁸⁺ octahedron^{13,16} and close to the positions of the bands in VO(PO₃)₂. The infrared transmission spectrum (KBr disk) reveals a band at 951 cm⁻¹ attributable to V-O stretching and other bands at 782, 807, 1,030, 1,113, 1,162, 1,230, 1,260 and 1,325 cm⁻¹. Bands at 682 cm⁻¹ and 710 cm⁻¹ observed¹⁷ in SiP₂O₇ and which are characteristic of octahedrally coordinated silicon¹⁸ are not observed here.

As is found in most tetravalent vanadium compounds, the V⁴⁺ ion in VO(P₂SiO₈) seems to be surrounded by an octahedron of oxygen atoms, but displaced towards one oxygen with the formation of one short and one long V-O bond. In VOSO₄·5H₂O (ref. 19) and VOSO₄·3H₂O (ref. 20) and many vanadyl complexes¹², the VO₆ octahedra are isolated from one another, with the nearest neighbour oxygen bound only to the central vanadium and the remaining five oxygens being provided by the other groups present. In α - and β -VOSO₄ (refs 14, 21) and VOMoO₄ (ref. 22), the VO₆⁸⁺ octahedra are linked in infinite chains by the alternating long and short V-O bonds, with the four equatorial oxygens of each octahedron being provided by the XO₄ groups. The presence of magnetic ordering in VO(P₂SiO₈) indicates that the VO₆⁸⁺ octahedra cannot be totally isolated from each other, but the lower vanadium content relative to VOSO₄ and VOMoO₄ may entail a somewhat different arrangement of octahedra than is found in these compounds. It is perhaps surprising that magnetic ordering occurs at all in VOSO₄ and VO(P₂SiO₈), for there is only one unpaired electron per mag-

netic atom, and even in VOSO₄ which has the higher vanadium content, the VO₆⁸⁺ octahedra are directly linked in only one dimension. VO(PO₃)₂ which seems to have a structure containing isolated chains of corner linked VO₆⁸⁺ octahedra like VOSO₄, shows no evidence of magnetic ordering down to 1.5 K.

The formula of VO(P₂SiO₈) indicates that isolated PO₄³⁻ or SiO₄⁴⁻ ions are very unlikely to be present in the structure, and this material seems, therefore, to be the first reported condensed stoichiometric phosphosilicate. The stoichiometry (X₃O₈) of condensed tetrahedra is also unusual and we are not aware of the existence of any analogous silicate, M(IV)Si₃O₈, or phosphate, M(IV)P₃O₈. One possible way of linking the tetrahedra in such a situation which could also provide a basis for ordering of P and Si, is shown schematically in Fig. 1.

The ease of preparation of VO(P₂SiO₈) indicates that condensed phosphosilicates may be a more extensive class of materials than has been previously suspected. The preparation of other vanadium species, as well as phosphosilicates of other metals may be anticipated. New types of condensed tetrahedral framework may be formed, perhaps permitting the ability to tailor materials to obtain desirable properties.

We thank Professors R. M. Barrer and D. McConnell for discussions and Dr W. R. Robinson for pointing out the preparation of phosphosilicate zeolites. K. L. Tai assisted with the X-ray emission analysis and G. A. Pasteur measured the spectra. *Note added in proof:* C. M. Barrer and M. Liquornik²⁴ were unable to reproduce the results of Flanigen and Grose¹⁰ concerning the hydrothermal preparation of phosphosilicate zeolites.

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Measurement of tensile strength of liquids by an explosion technique

A WIDE variety of methods has been used in the estimation of the static tensile strength of water^{1,2}. Recently, a dynamic method, using an impulse-generated pressure wave, has been described which uses a measurement of the negative pressure produced by the wave reflected from a free surface³. Wilson *et al.*⁴ had previously shown how estimates of the negative-pressure wave reflecting from a free surface could be used to measure liquid tensile strength in underwater-explosion research, and an adaption of that technique has been used to



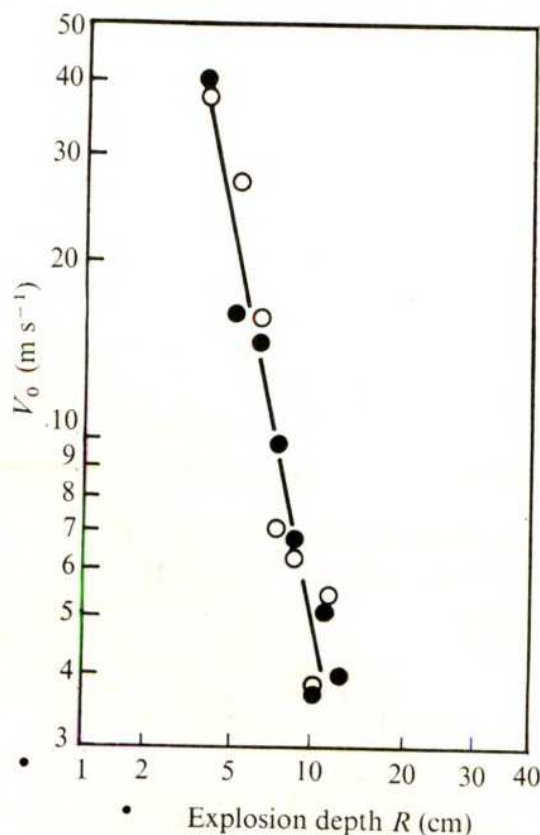
Fig. 1 Experimental set up for measuring liquid tensile strength by explosion technique.

estimate the tensile strength of water and glycerine. The basic idea is to make high speed motion picture photographs of the spray plume rising from an explosive charge detonated a small distance underwater. In a one-dimensional analysis the particle velocity of the explosive shock wave propagates as $P/\rho U$, and hence water at the free surface detaches with an initial velocity $2P/\rho U$, less the contribution of the tension exerted by the reflected shock wave $T/\rho U$. So the initial spray dome velocity is:

$$V_0 = (2/\rho U)(P - T/2)$$

where P is the maximum pressure of the explosion, T is the negative pressure in the reflected shock wave, ρ is the water density,

Fig. 2 Measured spray-dome velocity as a function of explosion depth. ●, Water; ○, glycerine.



and U is the shock wave velocity. By observing the value of V_0 at various explosive charge depths and extrapolating to zero velocity where $T = 2P$, an estimate of the water tensile strength can be made.

The experimental equipment is shown in Fig. 1, where the latter stage of a test is pictured. A small electrically actuated detonator containing 0.1 g of explosive was placed at depths from 2.5 to 12.7 cm below the surface in the centre of an open oil drum, filled to the top with the liquid under study. The only other equipment required is a high speed motion picture camera; we used a Photo Sonics camera at a speed of 500 frames s⁻¹. The displacement of the initial spray dome was measured from the film using an optical comparator. Typically, five or six frames per test contained the initial spray-dome rise data.

Figure 2 shows the results of the spray-dome velocity measurements as a function of charge depth, R . Extrapolation to the limit of measurement accuracy, 0.2 m s⁻¹, gives a value of R (for $V_0 \sim 0$) of 31 cm.

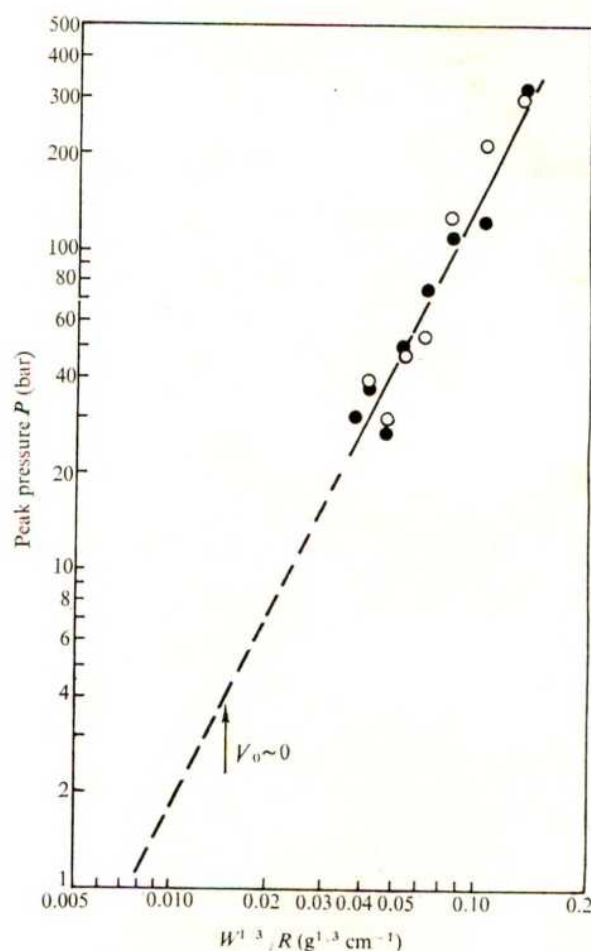


Fig. 3 Peak pressure as a function of explosive similarity parameter. ●, Water; ○, glycerine.

Figure 3 gives the peak pressure data plotted against the similarity parameter⁵ for explosives, $W^{1/3}/R$, where W is the charge weight and R the depth of the charge. The peak pressure, P , was obtained from the particle velocity through tables⁵ of the Rankine-Hugoniot equations. Using the extrapolated value of $W^{1/3}/R$ corresponding to $V_0 = 0$, an estimate of P and thus $T/2$ can be formed. We obtained a value of $T = 8$ bar, in good agreement with the result of Couzens and Trevena³, who obtained a value of 8.5 bar by direct measurement of the reflected wave. Both water and glycerine gave the same results.

This relatively simple way of estimating liquid tensile strengths may find application in comparative measurement of fluids such

as drag-reducing polymer solutions which cannot be subjected to the elaborate purification requirements of more conventional methods of measuring tensile strengths of liquids.

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Adaptive foci in protein evolution

THE *willistoni* group of *Drosophila* consists of several species, subspecies and semispecies endemic to the New World tropics¹. We have used electrophoretic techniques to study allelic variation at gene loci coding for enzymes in this group of species. Some 40 gene loci have been assayed in scores of natural populations belonging to 14 different taxa²⁻¹¹. Two remarkable patterns have emerged. First, within a given taxon the populations (sometimes geographically separated by thousands of kilometres) have quite similar configurations of allelic frequencies at nearly all loci; and second, the allelic configurations observed in different taxa show that any two species have quite similar genetic configurations at about half the loci, but very different configurations at (nearly) the other half. A similar situation obtains when infraspecific taxa are compared, except that the proportion of loci at which the two taxa are similar is more than half, and the proportion of loci at which they are totally different becomes much less than half.

The sets of loci at which any two taxa have similar genetic constitutions are different when different pairs of taxa are compared. At any one locus we find groups of two or more taxa genetically very similar to each other, but very different from the other groups; but the taxa that are similar at, say, locus A, may be different at locus B, whereas taxa that are different at locus A may be similar at locus

C, and so on. At most loci, two, three, or four different genetic configurations are found, with two or more taxa fitting each configuration.

We have suggested¹¹ that in the *willistoni* group of *Drosophila* the allelic frequencies cluster around a few orthogonally located 'adaptive peaks', or 'adaptive foci'. We also argued that this clustering could not be explained by the particular evolutionary history of the taxa (founder effects and bottlenecks in population size, phylogenetic relationships, and so on), as it is often the case that closely related taxa are in the same focus for some enzyme loci, but in different foci for other loci at which some of the taxa may share foci with less closely related species. Using the method of principal component analysis, we can provide support for both contentions.

Our analysis (Table 1) deals with seven species, six of which are morphologically almost indistinguishable. Two species consist each of two geographically separated subspecies; a third species consists of six semispecies or incipient species. In each of these 14 taxa we have analysed the allelic frequencies at the same 21 enzyme loci. The average number of genomes studied per locus is 15,036 for the whole group, or 1,074 per taxon. For each locus we have performed a principal component analysis in which taxa were taken as variables and allelic frequencies as cases. Geometrically, principal component analysis rotates Cartesian axes, maintaining orthogonality, in such a way that fewer axes are needed to explain the experimental variance. In our case, the original axes are frequencies of single alleles; the rotated axes are polymorphic combinations of the original axes (an unrotated axis remains monomorphic).

Table 1 shows, for each taxon at each locus, the single principal component which explains most of the experimental variance. There is a marked tendency for taxa to lie almost parallel to other taxa, so that usually several taxa lie on the same principal component. At the *Lap-5* locus, for example, seven taxa share principal component 1, four taxa share component 2, two taxa share component 3, and one taxon lies on component 4. The table indicates the proportion of the experimental variance explained by the principal components given. At the *Lap-5* locus, 98.3% of the experimental variance is explained by four principal components. Over all 21 loci, the average proportion of the experimental variance explained per locus is 95.0%. This is accomplished with an average of 2.9 principal components

Table 1 Principal components and proportion of variance explained for each of 21 gene loci in 14 taxa of the *D. willistoni* group. The mean number of alleles per locus is 12.8 ± 0.8

Loci	<i>t</i>	<i>ww</i>	<i>wq</i>	<i>ee</i>	<i>ec</i>	AM	IN	OR	AN	CA	T	<i>pv</i>	<i>i</i>	<i>n</i>	Percent explained of total variance
<i>Lap-5</i>	2	2	2	1	1	1	1	3	1	1	1	3	4	2	98.3
<i>Est-2</i>	1	2	1	2	1	1	1	1	1	1	1	3	4	3	97.0
<i>Est-4</i>	2	2	1	1	3	1	1	1	1	1	4	1	5	3	99.9
<i>Est-5</i>	2	1	1	1	3	1	1	1	1	1	1	1	2	2	99.9
<i>Est-7</i>	2	2	5	3	3	1	1	1	1	1	1	3	3	4	85.3
<i>Acph-1</i>	4	3	3	2	4	1	1	2	1	1	1	2	1	1	89.4
<i>Ald-1</i>	1	1	1	1	1	1	1	1	1	1	1	1	1	1	96.0
<i>Adh</i>	3	1	1	1	1	1	1	1	1	1	1	1	1	2	99.9
<i>Mdh-2</i>	3	2	2	1	4	1	1	1	1	1	1	1	3	4	86.9
<i>α-Gpdh</i>	1	1	1	1	1	1	1	1	1	1	1	1	1	1	98.0
<i>Idh</i>	1	1	1	1	1	1	1	1	1	1	1	1	1	1	99.6
<i>Me-2</i>	1	1	1	1	1	1	2	2	1	1	1	2	1	3	89.5
<i>Xdh</i>	3	1	3	1	1	1	1	1	1	1	1	1	2	2	77.3
<i>To</i>	1	1	1	1	1	1	1	1	1	1	1	1	2	2	92.0
<i>Tpi-2</i>	1	1	1	2	2	1	1	1	1	1	1	1	1	3	99.9
<i>Pgm-1</i>	2	1	1	2	2	1	1	1	1	1	1	1	2	3	99.9
<i>Adk-1</i>	1	1	1	1	1	1	1	1	1	1	1	1	2	3	99.3
<i>Adk-2</i>	1	1	1	2	2	1	1	1	1	1	1	1	2	2	99.5
<i>Hk-1</i>	2	2	2	2	3	1	1	1	1	1	1	1	3	1	90.1
<i>Hk-2</i>	1	1	1	1	1	1	1	1	1	1	1	1	1	1	98.0
<i>Hk-3</i>	1	1	1	1	1	2	2	2	1	1	1	1	3	1	99.8

* Symbols for the taxa: *t* = *D. tropicalis*; *ww* = *D. willistoni willistoni*; *wq* = *D. w. quechua*; *ee* = *D. equinoxialis equinoxialis*; *ec* = *D. e. caribbeensis*. *D. paulistorum* semispecies: AM = Amazonian; IN = Interior; OR = Orinocan; AN = Andean; CA = Centoamerican; T = Transitional; *pv* = *D. pavlovskiana*, *i* = *D. insularis*, *n* = *D. nebulosa*.

per locus. The original dimensionality (the number of axes or alleles) ranges from 8 to 21 with an average of 12.8 per locus. Table 1 also shows that the taxa that share principal components are different when different loci are compared, although more closely related taxa (like the six semispecies of *D. paulistorum*) tend to share principal components at more loci than less closely related taxa.

Our results indicate that, at each locus, natural selection favours one of a few alternative genetic configurations. With an average of 13 alleles per locus, we have a 13-dimensional genetic space for each locus. Yet all taxa fall within a few small foci (on average three) orthogonal to each other. These foci seem to be maintained by selection, whereas other combinations of alleles are not. Species evolve by changing their allelic frequencies so as to move rapidly from one adaptive focus to another, as evidenced by the fact that intermediate configurations rarely occur¹¹. Why the observed combinations of allelic frequencies are favoured in the *willistoni* group of *Drosophila* is a question for which we have no answer. It seems likely that selection may act on these loci as members of coadapted multilocus systems. Particular configurations of allelic frequencies occurring at some loci may affect the allelic configurations favourably selected at some other loci.

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Pre-Columbian purslane (*Portulaca oleracea* L.) in the New World

LIKE so many cosmopolitan weeds, purslane (*Portulaca oleracea* L.) has a history characterised by paradox and uncertainty. Most botanists have followed De Candolle¹ in assuming that it is native to the Old World and was introduced in the New World. There is, in fact, convincing historical evidence that the species was present in Europe before 1492 (ref. 2), and furthermore that it was introduced to North America in the seventeenth century³. On the other hand, the historical record also contains several anomalously early references to a wild purslane in the New World, and some botanists have argued that the species may have been present on both sides of the Atlantic before 1492 (ref. 4). Asa Gray even went so far as to suggest that the Vikings may have introduced it during their occupation of Greenland and Newfoundland⁵. We now present conclusive evidence that purslane was present in the New World in pre-Columbian times.

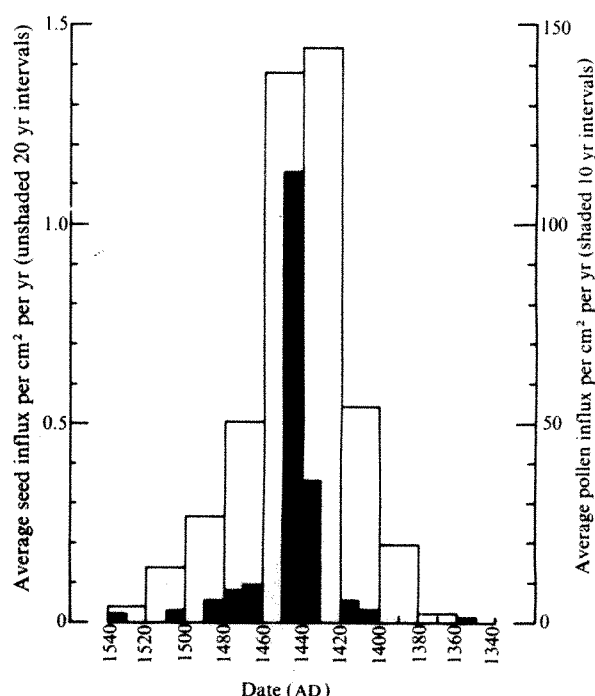
Purslane pollen and seeds were found in the sediments of Crawford Lake, which is some 35 km south-west of Toronto, Ontario. It has a surface area of 2.4 ha and a maximum

depth of 24 m. Largely because of its morphometry, there is an incomplete circulation in the water column (meromixis) and consequent anoxia in the bottom waters. The sediments are therefore free from disturbance by either water currents or both organisms. The sediments are varved, each varve consisting of a white summer layer rich in carbonate and a dark, winter layer rich in organic matter. Similar sediments have been described from other lakes in Ontario and adjacent New York State⁶⁻⁷. The significance of the varves in the present context is that they can be used to establish an accurate sediment chronology for the past 700 yr.

Sediment samples were obtained from the deepest part of the lake by freezing them *in situ* onto the outside surface of a 2-m long aluminium tube loaded with dry ice. The field and laboratory techniques used in sampling laminated sediments have been discussed in more detail by Swain⁸. Routine pollen analysis of contiguous 10-yr intervals indicated that purslane pollen was deposited in the lake during the period 1430-89 AD. More intensive analysis extended the range from 1350 to 1539 AD but not to the present. Figure 1 shows the pollen influx in absolute terms. The total pollen influx (arboreal pollen AP and non-arboreal pollen, NAP) into Crawford Lake during this period was approximately 3,500 grains per cm² per yr. Purslane pollen is relatively large and has a distinctive pericarpate arrangement of furrows (Fig. 2). It cannot be confused with any other pollen type in the local flora⁹. The identification was further confirmed by the discovery of purslane seeds in sediments from the same period. Ten samples (2.5 cm³) from contiguous 20-yr intervals were sieved through a No. 50 mesh (hole size 297 µm) and the residue was analysed for macrofossils. The seeds are generally well preserved, and are characterised by stellate epidermal cells. They correspond closely with modern Ontario herbarium material (Fig. 3). In terms of influx rates they show a similar if somewhat more regular trend than that of the pollen (Fig. 1).

The discovery of purslane pollen and seeds was unexpected. Apart from the problem of supposed Old World origins, the question arose as to how the fossils were deposited into the lake. Pollen dispersal through the atmosphere seemed unlikely as purslane is primarily a self-pollinating species¹⁰. Furthermore, the seeds, although small,

Fig. 1 Average pollen and seed influx.



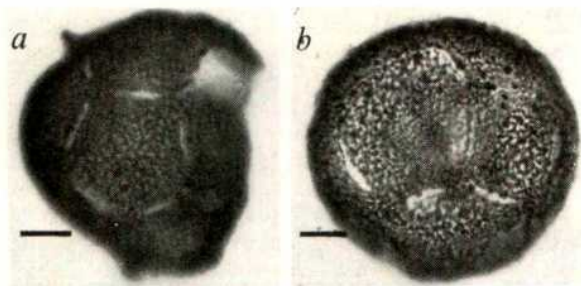


Fig. 2 Photomicrographs of *Portulaca oleracea* pollen grains. a, Fossil pollen grain (slightly broken) from Crawford Lake sediment dating 1430-39 AD. b, Modern pollen grain from southern Ontario. Scale bars represent 10 µm.

are not well adapted to dispersal by wind. Water transport also seemed unlikely as the lake is fed by a spring. The mechanism proposed here is that the pollen and seeds were deposited in the lake by man. More specifically, that agricultural Indians, who lived in the immediate vicinity of the lake, gathered purslane in their corn fields and washed it in the lake before eating it as a potherb. This washing would not only remove the soil from the plant but also release the pollen and seeds into the lake. That agricultural Indians were in the area at this time is also indicated by the presence of maize pollen (*Zea mays*) and sunflower pollen and seeds (*Helianthus annuus*) in the sediments dating from 1360 to 1650 AD. The fossils did in fact lead to the discovery of an Indian village site less than 1 km from the lake. The site is currently being excavated¹¹.

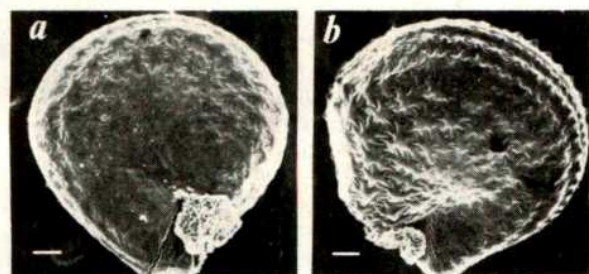


Fig. 3 Scanning electron micrographs of *Portulaca oleracea* seeds. a, Fossil seed from Crawford Lake sediment dating 1420-39 AD. b, Modern seed from southern Ontario. Scale bars represent 100 µm.

The discovery of purslane pollen and seeds throws new light on what until now have been rather puzzling aspects of the historical record. For example, as early as 1526 Oviedo included purslane in his list of plants native to both Hispaniola and Spain¹². In 1605 Champlain noted that purslane grew among the Indian corn fields near Plymouth, Massachusetts¹³. Similarly, Sagard, who lived with the Huron Indians during the years 1623 and 1624, reported that purslane was a common weed which grew naturally in fields among their corn and pumpkins¹⁴. Sagard's report is especially significant in that the Huron lived only about 150 km north of Crawford Lake, and were probably the direct descendants of the people who had been living at the Crawford Lake site 300 yr earlier¹⁵. As far as the species' presence in Ontario is concerned, it seems reasonable to assume that it came into the area with Indian agriculture.

Unfortunately, the broader question of ultimate origins cannot be answered on the basis of the evidence presented here. What is established is that the species was present on both sides of the Atlantic before 1492. The question is now raised as to how it achieved its wide distribution. One interesting possibility is pre-Columbian transfer by man. If this was the case, however, it must have been by a more southerly route than Asa Gray's Viking hypothesis suggests.

In Europe it does not grow north of latitude 60° and is absent from Iceland, Greenland and Newfoundland¹⁰. Low summer temperatures are probably the limiting factor here as successful germination requires air temperatures greater than 20 °C (ref. 10). A second, perhaps more plausible, explanation is natural dispersal. Purslane seeds are known to be eaten by birds and furthermore have a high viability rate after digestion¹⁰. They are small and presumably could have been carried either internally or externally. Even though purslane's pre-Columbian presence on both sides of the Atlantic may not be attributable to man, the species is still culturally interesting. Like the bottle gourd (*Lagenaria siceraria*), it was independently used in similar ways in both the Old World and the New.

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Suppressive effect of seminal plasma on lymphocyte activation

ALTHOUGH spermatozoa bear histocompatibility antigens (*H-2* in mice^{1,2} and *HL-A* in man^{3,4}) accelerated (immune) skin graft rejection is not known to occur in females previously multiply inseminated by a graft donor. The absence of detectable transplantation immunity in inseminated females correlates with the generally low immunogenicity of spermatozoa given by other routes. The decreased antigenicity of spermatozoa is not explained by a privileged immune status of the female reproductive tract as the vaginal route is adequate for immunisation against a variety of antigens⁵, especially if the tract is wounded⁶, and antibodies to spermatozoa decrease fertility in some situations⁷. Thus, it is probable that mechanisms have evolved to maintain a low immunogenicity of spermatozoa. What seems to be an exception to the low immunogenicity of spermatozoa—that antibody to a tissue-specific sperm antigen occurs naturally without deliberate immunisation^{8,9} and is readily elicited in a variety of species¹⁰—may represent a protective mechanism. Many substances present in seminal plasma may exert a suppressive effect by coating sperm membrane antigens or inducing immunosuppression by other means.

To investigate potential mechanisms for the observed low

Table 1 Human seminal plasma effects on PHA stimulation of peripheral blood lymphocytes

Seminal plasma (%)	No PHA		PHA stimulatory doses				250 µg ml ⁻¹		1,000 µg ml ⁻¹	
	c.p.m. ± s.e.m.	% Inhibition	c.p.m. ± s.e.m.	% Inhibition	c.p.m. ± s.e.m.	% Inhibition	c.p.m. ± s.e.m.	% Inhibition	c.p.m. ± s.e.m.	% Inhibition
0	707 ± 93	0	43,565 ± 3,724	0	185,494 ± 13,461	0	117,404 ± 14,611	0		
10%	202 ± 10*	72	2,325 ± 349†	95	71,606 ± 8,696†	62	59,587 ± 13,126*	50		
25%	292 ± 9*	59	15,007 ± 2,540*	66	181,642 ± 5,393	3	138,376 ± 6,317	0		
0.1%	867 ± 388	0	21,142 ± 4,167*	52	176,083 ± 6,188	6	127,674 ± 5,359	0		
0.001%	616 ± 150	13	31,999 ± 6,956	27	171,985 ± 9,325	8	116,210 ± 9,048	0		

Human peripheral blood lymphocytes from normal donors were purified by Ficoll-Hypaque density gradient centrifugation. Lymphocytes were cultured in triplicate in microtitre trays at concentrations of 2×10^5 cells per 0.2 ml RPMI 1640 + 10% pooled human serum. PHA-M (Difco) and serial dilutions of seminal plasma were added at $T=0$ and cultures were pulsed with ³H-thymidine from 66–72 h and collected on glass fibre filters with 0.9% NaCl using a MASH II Automatic Sample Harvester. DNA synthesis was determined by liquid scintillation counting in three separate experiments and is expressed as c.p.m. ± s.e.m.

* $P < 0.02$ against no seminal plasma.

† $P < 0.002$ against no seminal plasma.

immunogenicity of sperm, we have studied the effects of added spermatozoa or seminal plasma on two well defined *in vitro* assays for cellular immunity. First, the mixed leukocyte reaction (MLR) is a model for the afferent limb of natural alloimmunity and specifically detects lymphocyte-defined (LD) transplantation antigens which are genetically determined at the major histocompatibility locus¹⁰. Secondly, phytohaemagglutinin (PHA)-induced stimulation of lymphocyte DNA synthesis is predominately the result of proliferation of thymus-derived (T) cells and is widely employed as a model for antigen-induced lymphocyte transformation. The results demonstrate suppression of the induction of lymphocyte activation in both test systems by seminal plasma, and thereby, provide a partial explanation of the weak immunogenicity of ejaculates of spermatozoa.

Human seminal ejaculates were allowed to liquefy at 37°C for 30 min. Spermatozoa and hyaline bodies were centrifuged leaving a seminal plasma supernatant. The spermatozoa were resuspended in sterile saline, the hyaline bodies allowed to settle out, and the supernatant spermatozoa washed twice in sterile saline, counted in a haemocytometer and adjusted to the appropriate concentration. Mice (Jackson Laboratory, Bar Harbor, Maine) were maintained on normal laboratory feed. Sterile mouse spermatozoa were obtained by dissection of the ducti deferentes and epididymides of 2–8-month-old male mice. Tissue slices (1 mm) were suspended for several minutes in sterile saline, and the tissue fragments removed from the spermatozoa by a No. 16 gauze screen. Sperm were washed, counted in a haemocytometer and adjusted to the appropriate concentration in sterile saline. Dead spermatozoa were prepared by a rapid freeze-thaw cycle and a soluble sperm extract was prepared by taking the supernatant from a 100g, 10 min centrifugation of the frozen-thawed spermatozoa.

Human peripheral blood lymphocytes were isolated from

whole, heparinised blood drawn from normal donors using Ficoll-Hypaque gradient centrifugation¹¹. Murine splenocytes for culture were obtained by teasing the spleen with sterile forceps in Petri dishes. Cells were washed once in RPMI 1640 containing 5% (mouse) or 10% (human) heat-inactivated foetal calf serum, an antibiotic-antimycotic mixture and 100 mM L-glutamine. Responses to either PHA or allogenic cells in the MLR were determined by measuring DNA synthesis in responding lymphocytes.

Preliminary studies were performed to assess the effect of seminal plasma on the response of lymphocytes to PHA. Seminal plasma inhibited basal and PHA-induced DNA synthesis (Table 1), with much greater effects on PHA-containing cultures. The effect was partly dependent on both concentration of PHA and seminal plasma itself. The most marked suppression was detected at a suboptimal PHA dose (25 µg ml⁻¹) but was also noted at optimal (250 µg ml⁻¹) and supraoptimal (1,000 µg ml⁻¹) doses. At higher PHA doses the reduction in inhibition induced by seminal plasma might be explained by competition between the two substances for surface receptors on lymphocytes. We speculate that blockade of DNA synthesis in resting as well as activated lymphocytes may have resulted from prostaglandins present in seminal plasma¹². Prostaglandins have previously been shown to inhibit DNA synthesis in PHA-activated cells by raising intracellular cyclic AMP levels¹³.

We have also studied the effects of seminal plasma on the human MLR. A consistent inhibition by higher concentrations of seminal plasma on the MLR between unrelated donors is shown in Table 2. The single values of separate experiments are given since markedly different stimulation ratios occurred with genetically different donor pairs. Spermatozoa, however, had a variable effect on the human MLR, sometimes even stimulating it, a finding reported by others¹⁴. Since we did not determine the *HL-A* types of the lymphocyte and sperm donors, it was possible that spermatozoal *HL-A* antigens were stimulatory in

Table 2 Inhibition of human MLR by seminal plasma

% Seminal plasma in MLR	Exp.† 1		Exp.† 2		Exp.† 3	
	E/C*	% Inhibition	E/C	% Inhibition	E/C	% Inhibition
Control (no seminal plasma)	37.06 ± 14.89	—	4.22 ± 0.53	—	5.07 ± 0.32	—
0.25%	8.75	77	1.7	60	1.1	78
0.62%	57.00	0	2.9	31	4.3	16
1.25%	6.88	81	<1	100	<1	100
2.5%	6.21	83	1.4	67	1.5	70
6.25%	<1	100	<1	100	<1	100

*E/C = $\frac{\text{experimental}}{\text{control}} = \frac{(\text{c.p.m. human No. 1} \times \text{human No. 2 M})}{(\text{c.p.m. human No. 1} \times \text{human No. 1 M})}$; where M = mitomycin treated

Human lymphocytes (5×10^4) were mixed with 2×10^5 mitomycin-treated lymphocytes in 0.2 ml volume and the indicated amounts of seminal plasma added in 0.05 ml aliquots. The cells were cultured for 120 h, pulse-labelled with ³H thymidine for 6 h, and collected on glass fibre filters with the MASH II. DNA synthesis was determined by liquid scintillation spectrometry.

the MLR. It seemed unlikely that soluble *HL-A* antigens in seminal plasma¹⁵ could explain the inhibitory effects since the seminal plasma antigen would usually be allogeneic and would be expected to have a stimulatory, rather than an inhibitory, effect. On the other hand, it is possible that any potential stimulatory effect on the MLR of soluble *HL-A* antigens was overcome by the inhibitory effect of some factor(s) (such as prostaglandins) in seminal plasma. Alternatively, some of the nucleases¹⁶ or proteolytic activities¹⁷ of seminal plasma may be responsible for the inhibitory effect.

To elucidate the effects of spermatozoa on the MLR in mice, which provide a setting with defined histocompatibility genes, we used CBA/J (*H-2^k*) as the source of responding, and C57BL/6J (*H-2^b*) as the source of stimulating, splenocytes. Preliminary experiments using normal CBA/J splenocytes with mitomycin-treated C57BL/6J splenocytes revealed that DNA synthesis was maximal at 48 h culture and that a 1:1 ratio of stimulating to responding splenocytes gave optimal responses in the MLR. Both live and dead autologous spermatozoa showed statistically insignificant and slight inhibitory effects at low dosages of spermatozoa on the MLR in this *H-2* combination, whereas the soluble sperm supernatant had no effect. Thus, the inhibitory effect of seminal plasma is unlikely to be the result of soluble components of spermatozoa in the seminal plasma.

The inhibitory effect of seminal plasma may be partly responsible for the poor immunological response in males to genital infection with cytomegalovirus¹⁸ or to first infections with *Neisseria gonorrhea*¹⁹. The inhibitory effect of seminal plasma components on the MLR in mice is unlikely to explain the necessity for the large numbers of spermatozoa required to affect decreased fertility in immunised females²⁰⁻²² since epididymal spermatozoa, free of seminal plasma, were used for those experiments. Finally, we conclude that the low immunogenicity of inseminated sperm may be aided by the presence of inhibitory factors present in seminal plasma. A likely candidate is prostaglandin, known to be present in high concentrations¹² and previously shown to exert suppressive effects on lymphocyte activation through cyclic AMP-mediated mechanisms¹³.

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Direct transformation of 3T3 cells by Abelson murine leukaemia virus

MURINE leukaemia viruses (MLV) cause tumours of haemopoietic origin *in vivo*^{1,2}, but although they replicate *in vitro*, they generally do not transform cells. MLV provides helper activity³ for the replication of defective murine sarcoma virus (MSV)⁴ which possess a transforming activity⁵. Abelson and Rabstein⁶ isolated an agent in association with Moloney leukaemia virus (MLV-M) which causes a rapidly progressive lymphoblastic leukaemia of bone-marrow-derived lymphocytes (B cells)⁷. In addition, this Abelson virus (MLV-A) in conjunction with MLV-M causes rapid appearance of immunoglobulin-producing plasmacytomas in BALB/c mice primed with oil⁸. We now report that MLV-A can be defective for virus replication and show that this agent directly transforms 3T3 cells *in vitro*. A quantitative transformation assay is described.

We found that cell-free filtrates of Abelson tumour extracts from BALB/c or Swiss mice consistently transformed NIH/3T3 cells. Transformed foci appeared 3-5 d after inoculation, and were recognised by the presence of rounded and elongated refractile cells separated by cells of normal appearance. Clones of transformed cells (isolated with the use of cloning cylinders) had saturation densities 3-6 times greater than that of untransformed NIH/3T3, demonstrating the loss of density-dependent inhibition of cell division. Also, unlike NIH/3T3, many transformed cells formed colonies in a soft agar gel. Tissue culture fluids overlying cultures of transformed NIH/3T3 cells were used as a source of virus stock for subsequent experiments.

The Abelson leukaemia virus causes the B-cell leukaemia equally well in NIH/Swiss and BALB/c mice⁶, and thus the agent is NB-tropic. To demonstrate the tropism of the transforming activity *in vitro*, we simultaneously infected NIH/3T3 and BALB/c-3T3 cells with serial dilutions of a virus stock. Transforming activity was NB-tropic, for there was only a twofold difference in the frequency of transformation in the two cell lines (Table 1); a tenfold or greater difference would

Table 1 Tropism of the transforming activity

Virus stock dilution*	NIH/3T3		BALB/c-3T3	
	Transformed foci	XC cell syncytia	Transformed foci	XC cell syncytia
1 × 10 ²	79	TMC	31	TMC
3 × 10 ²	17	TMC	8	TMC
1 × 10 ³	5	TMC	3	TMC
3 × 10 ³	1	TMC	0	TMC
1 × 10 ⁴	0	TMC	0	TMC
3 × 10 ⁴	0	34	0	41
1 × 10 ⁵	0	15	0	8
3 × 10 ⁵	0	4	0	3
1 × 10 ⁶	0	1	0	1
Mock infected	0	0	0	0

NIH/3T3 (clone 1)¹² and BALB/c-3T3 (clone A31)¹³ were maintained in Dulbecco's modified Eagle's MEM containing 4.5 g l⁻¹ of glucose and 10% calf serum (Colorado Serum Co.). One day before inoculation, a culture was trypsinised and 10⁵ cells were seeded in replicate 60 mm diameter plastic dishes (Falcon) in medium containing 8 µg ml⁻¹ of Polybrene¹⁴ (Aldrich). The tissue culture medium overlying virus-transformed NIH/3T3 cells was used as a source of virus stock. These stocks were filtered (pore size, 0.45 µm) immediately before use, and serial dilutions of 0.5 ml were used for inoculation. The medium was changed on the fifth day after inoculation. Transformed foci were scored visually on the tenth day on unstained preparations using an inverted microscope at ×100 magnification. The cells were then irradiated with ultraviolet light and overlaid with XC¹⁵ cells. Foci of MLV-M replication were detected as syncytial plaques¹⁶, and scored 3 d later.

*Expressed as the reciprocal of the dilution.
TMC, too many syncytia to count accurately.

Table 2 Inhibition of transformation by anti-MLV-M serum

Virus stock dilution	Anti-MLV-M rat serum		Normal rat serum	
	Transformed foci	XC cell syncytia	Transformed foci	XC cell syncytia
10 ¹	1	TMC	TMC	TMC
10 ²	0	TMC	67	TMC
10 ³	0	22	5	TMC
10 ⁴	0	2	0	TMC
10 ⁵	0	0	0	8
10 ⁶	0	0	0	0
Mock infected	0	0	0	0

Anti-MLV-M serum was prepared in rats bearing tumours caused by the MSV(MLV-M) complex. The serum neutralised MLV-M at a 1:160 dilution but did not neutralise Gross virus past a 1:20 dilution. A 1:40 dilution of anti-MLV-M serum or normal rat serum was incubated with previously filtered virus stock at 25 °C for 30 min. The transforming activity and MLV-M titre were determined in clonal BALB/c-3T3 as described in Table 1.

be expected for a virus with only N or B tropism⁹. The titre of replicating MLV-M in the stock, detected by the XC cell test, was nearly identical on N and B cells and was higher than that of transforming activity. The number of transformed foci was inversely proportional to virus dilution, suggesting

the untransformed culture that gave the positive XC cell test developed the thymic tumours of Moloney leukaemia after 3 months.

Thus MLV-A can replicate in tissue culture and seems to cause morphological transformation *in vitro*. To confirm that MLV-A has transforming activity, we isolated a clone of virus-transformed cells (termed ANN-1) that failed to secrete XC plaque-forming virus. Tissue culture fluids prepared from ANN-1 neither transformed NIH/3T3 cells nor caused Abelson leukaemia when inoculated into mice (Table 4). As no C-type particles were observed by electron microscopy and as tissue culture fluids were free of viral reverse transcriptase, this permanent clonal line is termed a non-producer¹¹. The ANN-1 line is tumorigenic, for 1×10^6 cells readily cause tumours (fibrosarcomas) when inoculated intraperitoneally into newborn Swiss mice. A clone of MLV-M, isolated independently of MLV-A, was used to infect the ANN-1 cells. The infected cells produced an agent that transformed NIH/3T3 and caused Abelson leukaemia (Table 4). Gross virus also rescued MLV-A. An independently isolated non-producer also replicated a transforming virus that caused Abelson leukaemia virus after infection with MLV-M. Thus MLV-A has a transforming activity and can be defective for virus replication. Although the mechanism of leukaemogenesis remains unclear, it is likely that the transforming activity of MLV-A is needed to cause the murine B-cell leukaemia.

Table 3 End point dilution of the transforming and leukaemogenic activities of MLV-A

Virus stock dilution	Titre		Properties of mass culture		Abelson leukaemia No. with leukaemia/ No. of survivors
	Transformed foci	XC cell syncytia	Morphological transformation	XC cell test	
1 × 10 ⁴	70	TMC	+	+	6/6
3 × 10 ⁴	28	TMC	+	+	ND
1 × 10 ⁵	17	40	+	+	6/8
3 × 10 ⁵	2	5	+	+	7/10
1 × 10 ⁶	0	2	—	+	0/21*
3 × 10 ⁶	0	0	—	—	0/8

Clonal NIH/3T3 cells were infected with a tissue culture preparation of virus stock as described in Table 1. Plaques of XC syncytia were determined on two plates at each dilution 10 d after the adsorption period. Transformed foci were scored 15 d after the adsorption period on two plates; 15 d later cells from the latter plates, at each virus dilution, were pooled and grown to mass culture. The cultures were examined for the presence of transformed cells, and a subculture of each was overlaid with XC cells to detect replicating MLV-M. Media overlying the mass cultures was filtered (pore size, 0.45 µm) and 0.1 ml was inoculated intraperitoneally into Swiss mice before the second day of life. Three to four weeks later, the animals were killed and autopsied, and the number with leukaemia was compared with the number of survivors. Animals scored as having Abelson leukaemia had gross and histological evidence of lymphoblastic infiltration of the marrow, lymph nodes and meninges, with no involvement of the thymus⁶.

*Sixteen animals developed thymic tumours, which were detected 3 months after inoculation¹.
ND, not determined.

that one agent alters the growth properties of these cells. MSV similarly transforms some permanent cell lines with one-hit kinetics¹⁰.

Anti-MLV-M serum inhibited the transforming activity of virus stock 500-fold and reduced the number of XC plaques 40-fold (Table 2), suggesting that the focus forming agent has one or more coat proteins in common with MLV-M.

To determine whether leukaemogenic activity correlates with transforming activity, we used the end point dilution method. NIH/3T3 cells were infected with serial dilutions of virus stock. Transformed foci were absent when the dilution exceeded 3×10^5 , while a few XC plaques were present at 1×10^6 (Table 3). Cells from the plates at each dilution were pooled and grown to mass culture to provide virus for inoculation of animals. The mass cultures at 3×10^5 or less were largely composed of transformed cells; the cells from the 1×10^6 dilution were not transformed, but gave a positive XC test. Only the animals inoculated with the virus prepared from the transformed cells developed Abelson leukaemia, which was noted at 1 month. Although virus prepared from untransformed cultures did not cause this disease, 80% of animals inoculated with virus from

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Table 4 Rescue of MLV-A with MLV-M

Cell line	Addition of MLV-M	Replication of transforming activity	Abelson leukaemia No. with leukaemia/ No. of survivors
ANN-1	No	—	0/19
	Yes	+	17/21
NIH/3T3	No	—	0/6
	Yes	—	0/9

The ANN-1 (Abelson-NIH/3T3-Nonproducer) clonal cell line was isolated by infecting NIH/3T3 cells with a 1×10^7 dilution of virus stock; MLV-M could not be detected at a 3×10^6 dilution. The replication of the transforming and leukaemogenic activities were assayed as described in Table 3.

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In vitro expression of erythroid differentiation induced by Friend polycythaemia virus

WITHIN a few days after infection of susceptible mice with the Friend polycythaemia virus (FVP), a large increase in red cell production occurs leading to polycythaemia¹⁻³. The mechanism by which FVP induces this effect is not known although it has been shown that this induction does not result from the presence of erythropoietin (Ep) in the inoculum and is not dependent on the endogenous production of Ep (refs 1 and 3). As yet, no system for both infection *in vivo* and subsequent expression of erythroid differentiation *in vitro* has been described. We have developed a method whereby infection of spleen cells can be accomplished *in vivo* and the late events of erythroid differentiation can be studied *in vitro*. We report that spleen cells cultured soon after infection *in vivo*, before increases in haemoglobin synthesis are detectable, will later increase their rate of haem, or haemoglobin, synthesis *in vitro*. The effect of Ep on the FVP-induced erythroid differentiation was also tested.

Ex-hypoxic plethoric BALB/c mice were used in all experiments. Mice treated in this way have extremely low levels of splenic erythropoiesis⁴. FVP or Ep was administered to the mice at various times before the preparation of spleen cell suspensions for cell culture. The initial rate of ⁵⁹Fe incorporation into haem during the first 4 h of cell culture was used as an indicator of ongoing erythropoiesis. As a measure of expression of the erythroid differentiation *in vitro*, ⁵⁹Fe incorporation into haem was determined after 2 d of cell incubation^{5,6}. Haem radioactivity after the addition of Ep to marrow cells *in vitro* has been shown to be equivalent to haemoglobin radioactivity⁷, and replicate experiments have shown a similar equivalence for mouse spleen cells *in vitro* after FVP infection (W.D.H., and S.B.K., unpublished).

Splenic cell suspensions prepared up to 48 h after infection with FVP (FVP-cells) exhibited a low rate of haem synthesis during the initial 4 h of incubation (Fig. 1a). Additional studies have shown that a large increase in the initial rate of haem synthesis is manifest 56 h after FVP infection at the virus dose employed in these experiments. Although the initial rate of haem synthesis by 0-48 h FVP-cells remained quite low, the amount of ⁵⁹Fe incorporation increased markedly when these cells were subsequently incubated for 48 h *in vitro* (Fig. 1b).

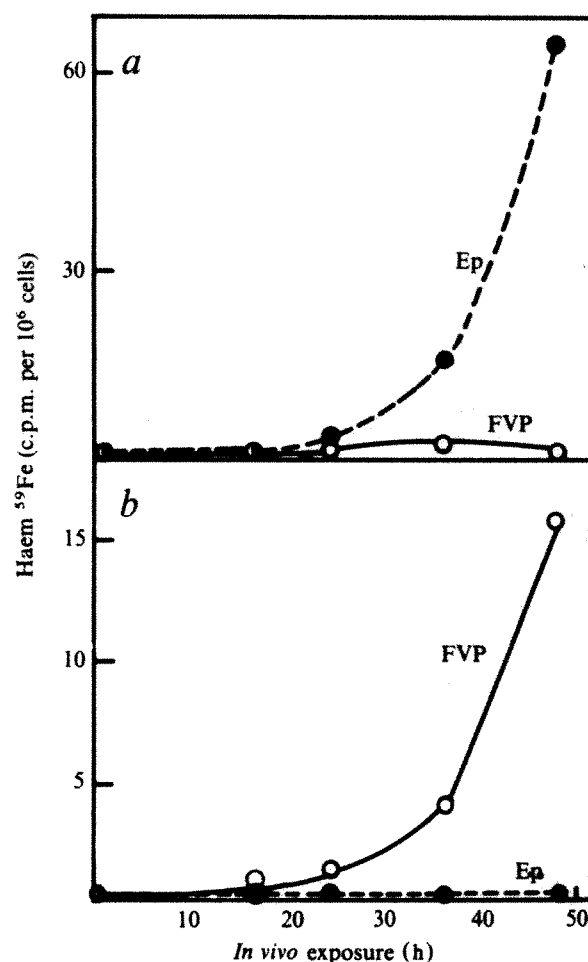


Fig. 1 Effect of FVP or Ep *in vivo* on initial (a, 0-4 h ⁵⁹Fe pulse) and late (b, 44-48 h ⁵⁹Fe pulse) rate of haem synthesis by spleen cells *in vitro*. All cell suspensions were prepared and cultures initiated simultaneously. At 16, 24, 36 or 48 h before the initiation of the cultures mice were injected intravenously with 50 μ l FVP plasma containing 80,000 focus forming units³ diluted to 0.2 ml with Hanks' solution, or subcutaneously with 3 U of erythropoietin. The zero time controls did not receive FVP or Ep. Spleen cell suspensions were prepared by repeated perfusion of the spleens with Hanks' solution. The cells were collected by centrifugation and washed twice with Hanks' solution. The washed cells were then resuspended in an incubation medium containing 3 U ml⁻¹ heparin, 30 U ml⁻¹ penicillin, and 2 μ g ml⁻¹ streptomycin, and consisting of 60% NCTC-109, 20% human AB plasma and 20% newborn calf serum. Each cell suspension represents the spleen cells from two mice. The cell suspension (1 ml) containing 12×10^6 - 16×10^6 nucleated cells ml⁻¹ was pipetted into Falcon Plastics 35 \times 20 mm tissue culture Petri dishes and incubated at 37 $^{\circ}$ C in an atmosphere of O₂-CO₂ (95%:5%). At 0-4 h (a) or 44-48 h, (b) 1 μ Ci ⁵⁹Fe, in 0.1 ml medium, consisting of 40% iron-free human AB plasma and 60% NCTC-109, was added to the cells. After 4 h the cells were collected and lysed in dilute Drabkin's solution⁸. Haem was extracted with cyclohexanone and the radioactivity was determined in a gas-flow Geiger-counter with a background of 1-2 c.p.m. as previously described⁶. Each point is the mean of duplicate cultures.

The level of haem synthesis attained in cultures of infected cells increased as the time of exposure to FVP *in vivo* was lengthened. This could be the result of an increased period of infection or an increase in the total time allowing visualisation of a delayed effect. For comparison, cell cultures were also prepared from the spleens of mice treated at varying intervals with Ep (Ep-cells). With these cells, increases in the initial rate of haem synthesis *in vitro* were observed as early as 24 h after the administration of Ep *in vivo* (Fig. 1a). The erythropoietic stimulation by the hormone was much more apparent after 36 and 48 h. Other investigators have noted that increases in splenic erythropoiesis are observed earlier after Ep administration than

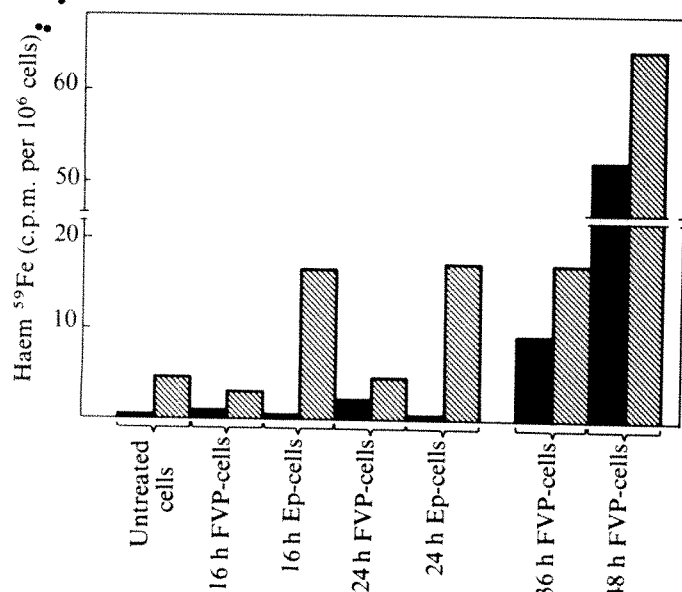


Fig. 2 Effect of FVP and Ep *in vivo* on capacity of cells to respond to Ep *in vitro*. The pretreatment of mice and cell incubation conditions were as described in Fig. 1. Ep (0.48 U ml^{-1}) was added at the time the cultures were started (hatched columns). Controls had no added Ep (solid columns). ^{59}Fe ($1 \mu\text{Ci}$) was added to the plates after 30 h of culture. Sixteen hours later the cells were collected and haem was extracted as in Fig. 1. Each bar is the mean of duplicate cultures.

after administration of FVP (ref. 2). Whether this temporal difference reflects a longer latent period for FVP induction of erythropoiesis or is related to the doses of FVP or Ep used is not known. In contrast to the FVP-cells the rate of synthesis by the Ep-cells after 48 h of cell culture had declined to control levels (Fig. 1b). Experiments are in progress to determine whether the Ep-cells exhibit increased haem synthesis at an earlier point in the culture period.

These experiments indicate that although the rate of haem synthesis has not been altered within 16–24 h after infection of mice with FVP, the spleen cells can increase haem synthesis 48 h later *in vitro*. If virus-induced erythropoiesis is viewed as a process in which cells are infected and induced to undergo erythroid differentiation^{1–3,8} then this system enables the separation of the early events involved in this process from the very late events involved in haemoglobin synthesis. The development of a system in which infection of spleen cells is accomplished *in vivo* and the onset of haemoglobin synthesis is manifested *in vitro* should facilitate the analysis of the biochemical events mediating the virus-induced polycythemia.

Spleen cells from plethoric mice treated with FVP were also cultured for 46 h in the presence or absence of the hormone, and ^{59}Fe was added to the cells during the last 16 h of cell incubation. In all cases, cultures containing the hormone had higher rates of haem synthesis (Fig. 2). McGarry and Mirand⁹ have previously shown that mouse erythropoiesis *in vivo* was still responsive to Ep early after infection, but the response was lost after several days. Plethoric mice were also treated with Ep 16 and 24 h before placing the spleen cells into cell incubation with and without the hormone (Fig. 2). After 46 h of cell culture without Ep, the cells treated with Ep *in vivo* synthesised only slightly more haem than control cells. Whereas the Ep-cells exhibited a potentiated response to Ep *in vitro* that was much larger than the response of control cells and was similar to the potentiated response observed *in vivo*^{2,10}, the FVP-cells did not show this type of response. Previous treatment with either Ep or FVP *in vivo* produces erythroid differentiation, but these experiments show that a large difference in the two cell populations is manifest when they are incubated with Ep *in vitro*. Ep has been shown to act on the Ep-responsive cells to stimulate their proliferation and increase their number^{11,12}. This could account for the potentiating effect of one dose of Ep on a second, later dose. In this context we conclude that FVP infection does

not produce an increase in similar Ep-responsive cells in the same time as Ep.

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Spectral sensitivity and intensity thresholds in *Nasonia* photoperiodic clock

FEMALES of the parasitic wasp *Nasonia vitripennis* give rise to diapausing progeny (fourth instar larvae) when maintained at short daylength ($< 15.25 \text{ h/24}$) but to continuously-developing or non-diapause progeny at long daylength ($> 15.25 \text{ h/24}$) (ref. 1). Although the precise anatomical location of this photoperiodic 'clock' has not been determined, it is entirely maternal since the eggs are committed for either diapause or non-diapause development before they are deposited within the host blowfly puparium: by analogy with the aphid *Megoura viciae*² and the silkworm *Antheraea pernyi*³, the clock and photoreceptors are probably in the brain. Photoperiodic time measurement in *N. vitripennis* is known to be a function of the circadian system⁴, and in all probability two separate circadian oscillators, one phase-set by the 'on' transition of the photoperiod and one by the 'off' transition, serve to 'measure' the phase-angle between 'dawn' and 'dusk'⁵. Since, in principle, an experimentally observed action spectrum should correspond directly with the absorption spectrum of the pigment molecule involved and thereby lead to its identification, the spectral sensitivities of the 'dawn' and 'dusk' transitions of the daily photoperiod were determined for *N. vitripennis*.

The ability of insects to discriminate between long and short-day photoperiods using light of different wavelengths has often been studied. Most earlier work, however, was restricted to different wavelengths with no regard to intensity^{6–10}, or to constant light energies at all wavelengths^{11–13}, both of these providing limited information on maximum sensitivities. Indeed, properly conducted energy-compensated action spectra for the photoperiodic response in insects have been determined on very few occasions^{14–17}. Many of these studies have shown an insensitivity to red light^{6–12,14–16}. On the other hand, a number of insects, including the knot grass moth *Acronycta rumicis*⁸, the Colorado potato beetle *Leptinotarsa decemlineata*¹³, the pink boll-worm moth *Pectinophora gossypiella*¹⁸ and the Ichneumonid *Pimpla instigator*¹⁹, are able to respond to wavelengths well into the red end of the spectrum (655–700 nm); and females of *N. vitripennis* have been shown to discriminate between short and long daylengths of red light above 600 nm (ref. 5). Bradshaw¹⁷, working with the reactivation of diapausing larvae of the phantom midge *Chaoborus americanus*, has also shown a maximal sensitivity at longer wavelengths (540 nm)

and a considerable response to red. Evidently, the pigment molecules involved in the insect photoperiodic response absorb maximally in quite different regions of the visible spectrum, and are consequently different in different insect species.

Figure 1 shows that the action spectra for the 'dusk' and 'dawn' transitions in *N. vitripennis* are similar with maximum sensitivity between 554 and 586 nm and considerable sensitivity extending into the red at 617 nm. For the 'dawn' transition (Fig. 1b) the insects responded to about $0.04 \mu\text{W cm}^{-2}$ at 554 nm, 586 nm and 617 nm, and the calculated threshold at 586 nm was in the region of $0.02 \mu\text{W cm}^{-2}$. The threshold at 653 nm was about $0.95 \mu\text{W cm}^{-2}$ but the insects failed to respond to pulses in excess of $30 \mu\text{W cm}^{-2}$ at 765 nm. Females of *N. vitripennis* were also about 20 times more sensitive to a pulse (586 nm) at 'dawn' ($0.02 \mu\text{W cm}^{-2}$) than to one at 'dusk' ($0.46 \mu\text{W cm}^{-2}$).

These action spectra are quite different from those previously described for the photoperiodic reaction. The work of Lees¹⁴ with the aphid *M. viciae*, for example, showed a maximal sensitivity to a 1-h light pulse placed 1.5 h after the end of the 13.5-h main photoperiod in the blue region of the spectrum (450–470 nm). At this wavelength, threshold values (50% virginopara-production) occurred at an intensity of about $0.2 \mu\text{W cm}^{-2}$. Above about 490 nm there was a rapid decline in sensitivity and very little response in the red. Similarly, in the codling moth *Carpocapsa pomonella* and the oak silkworm

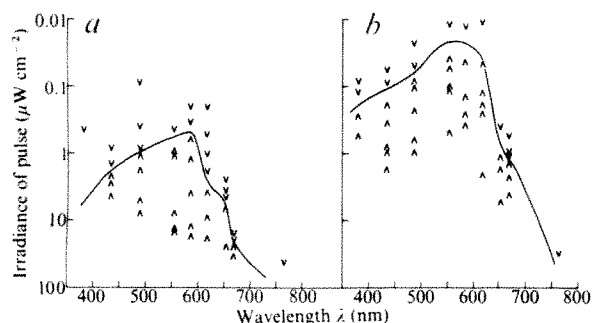


Fig. 1 Action spectra for the 'dusk' (a) and 'dawn' (b) transitions of the daily photoperiod in *N. vitripennis*, monochromatic light of different intensities being offered as a 3-h pulse just after, or just before, a 13 h 'main' light period in each 24 h cycle. ▼, Groups in which more than 50% of the wasps produced diapausing progeny; ▲, groups in which less than 50% of the wasps produced diapausing progeny. The curve connects those light intensities required to produce a threshold response (50% diapause-producers) in groups of females exposed to wavelengths between 380 and 765 nm. Most groups consisted of 60 female wasps. Groups of *N. vitripennis* were enclosed in glass jars with an ample number of host fly puparia (*Sarcophaga argyrostoma*) in artificial light cycles and at 18 °C for 15 d. At the end of this period, 60 of the surviving female wasps in each group were separated and provided with two fresh hosts for a further 2 d. These puparia, now parasitised, were then removed and incubated at 25 °C in the dark for a further 10 d before being opened to ascertain the diapause or non-diapause status of the progeny. The adult insects were exposed daily to a short-day photoperiod of 13 h white light ($240 \mu\text{W cm}^{-2}$) and 11 h darkness (LD 13:11), supplemented with 3 h of monochromatic light at either the beginning (dawn) or at the end (dusk) of the main photoperiod. The rationale behind this approach was that if the insects saw the supplementary coloured light they would react to the long-day regime (LD 16:8) so produced, and give rise to non-diapause progeny, whereas if they did not see this additional light they would merely respond to LD 13:11 and produce their offspring in diapause. Control groups of LD 13:11 and LD 16:8 (white light) were used. Monochromatic light of different intensities was obtained with the aid of projectors fitted with Osram 6 V 15 W microscope lamps and a series of Balzer narrow-band interference filters coupled with neutral-density filters. The distance between the bulb filament and the experimental insects was about 30 cm. At each wavelength interval the intensity of the light pulse was systematically altered in different experiments until a threshold response (50% of the females producing diapausing progeny) was obtained. The irradiance of each light pulse was measured with a Tektronix digital radiometer in $\mu\text{W cm}^{-2}$.

Antheraea pernyi^{15,16} the action spectra for breaking diapause showed maximum sensitivity between 400 and 500 nm at thresholds between 0.02 and $0.03 \mu\text{W cm}^{-2}$. Therefore, although the intensity thresholds measured here are similar, the pigment in *N. vitripennis* absorbs in quite a different region of the spectrum. In *C. americanus*¹⁷, on the other hand, the maximal response was at 540 nm, closer to that for *N. vitripennis*, but the intensity threshold was considerably lower ($0.001 \mu\text{W cm}^{-2}$).

Since photoperiodic time measurement in *N. vitripennis* involves circadian oscillators^{4,5}, it is likely (as with other circadian systems) that light pulses falling in the early part of the night cause phase delays ($-\Delta\Phi$) whereas pulses falling in the latter part of the night cause phase advances ($+\Delta\Phi$). It is interesting, therefore, to compare the present data with the known action spectra for overt circadian rhythms. The action spectra for the initiation of the rhythm of egg hatch in *P. gossypiella*²⁰ and those for advance and delay phase shifts of the rhythm of pupal eclosion in *Drosophila pseudoobscura*²¹ are all similar, with a peak of sensitivity at 420–480 nm. These action spectra are quite different from those for the photoperiodic response reported here for *N. vitripennis*.

Finally, the low intensity thresholds measured here raise ecological questions concerning the duration of the effective photoperiod and whether moonlight could 'upset' long-night measurement under certain conditions. In *N. vitripennis* the dawn and dusk thresholds occur at 0.02 and $0.46 \mu\text{W cm}^{-2}$, respectively. Bünning²², working in Tübingen (48°N), close to the vernal equinox (March 1–12, 1969), showed that these values occurred approximately 45 min before sunrise (and probably 45 min after sunset). For *N. vitripennis*, therefore, the effective daylength at this time of the year must be regarded as about 13 h 20 min rather than the 11 h 52 min between sunrise and sunset. Similar calculations should be carried out for effective daylengths at other times of the year. As far as moonlight is concerned it is interesting that the dawn threshold is lower than the intensities reached by full moonlight (0.04 – $0.28 \mu\text{W cm}^{-2}$)²³. Interference by moonlight, however, is considered unlikely because photoperiodic thresholds are generally correlated with the ecological habitats and habits of the sensitive stages, those insects in 'cryptic' or shaded situations having a lower threshold than those which are exposed²³. Furthermore, it is known that 8 to 13 long-day cycles are required to reverse the induction of diapause in *N. vitripennis*²⁴ and—because of the period difference between the lunar day (24.8 h) and the solar day (24.0 h)—this number of lunar interruptions is unlikely to occur in the latter half of the night before the period of brightest moonlight encroaches on sunrise and no longer functions as a perturbation.

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Influence of differential chromosome spiralisation on karyotype morphology

THE existence of differential chromosome contraction¹⁻³ presents difficulties for morphometric chromosome analysis. Spiralisation rates of all the chromosomes change simultaneously⁴, and the sizes of all the chromosomes in the set must be correlated with one another, and also with the extent of contraction; the latter may be expressed as the total complement length (the total length of the chromosome complement).

We have measured chromosomes of *Allium cepa* (chromosome complement 52), *Allium fistulosum* (50) and *Macaca mulatta* (28 for female and 23 for male). Since the relationship between chromosome (and arm) length depends on the total complement length, we plotted the absolute length of every identifiable chromosome and its arms, against the total complement length (Fig. 1). Amalgamation of the lines obtained (Fig. 2) shows that there is a linear relationship between the absolute length of each chromosome (or its arm) and the total complement length.

Each linear plot for a chromosome (or arm) has its own angle of slope (α) but this does not seem to depend on the chromosome (arm) length. For any chromosome set, therefore, the mathematical expectation for the size of each chromosome can be determined by measuring any other⁵. This makes it possible to reveal some tiny aberration which cannot be observed by the routine pair or polykaryogram methods, and nomogram chromosome analysis can also be performed.

The correlation between relative chromosome length (ratio

Fig. 1 Correlation between *Macaca* chromosome I absolute length and I total complement length. 1s, short arm; 1l, long arm; 1, whole chromosome. All graphs were smoothed by a graphic method. The root tips of the onion seeds were pretreated with 0.025% colchicine for 2 h, then with saturated solution of 8-oxyquinoline for 2 h, fixed in acetic acid/alcohol 1:3, stained using the Feulgen method, macerated using cytase and squashed in 45% acetic acid. The cover-slides were separated by the usual dry-ice method and the preparations mounted in Canada balsam. The preparations of the monkey chromosomes were obtained by the Moorhead method with modifications. Chromosomes were measured on the positive prints with magnification $\times 3,400$ (onions) and $\times 3,200$ (monkey) using a glass ruler calibrated in 0.1 mm steps and a stereomicroscope.

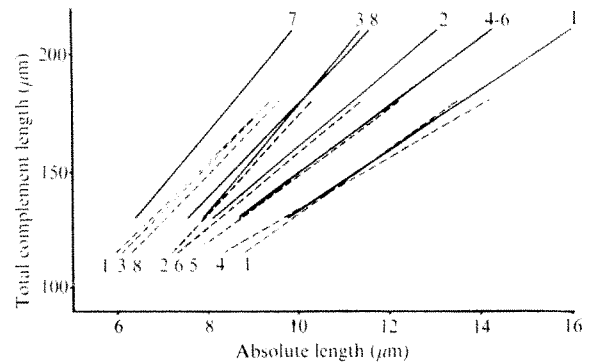
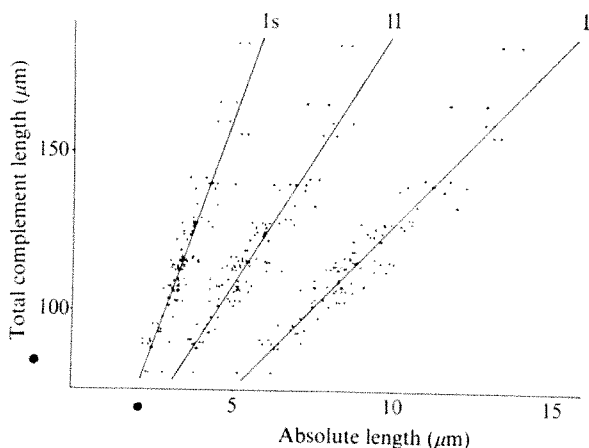


Fig. 2 Dependence of absolute lengths of *A. cepa* (solid line) and *A. fistulosum* (dotted line) chromosomes on total complement length. 1-8, chromosome numbers.

of the chromosome length to the length of complement) and the extent of spiralisation is hyperbolic:

$$l_{rel} = \frac{l_i}{L_i} = \frac{(l_{min} + (L_i - L_{min}) \cot \alpha)}{L_i}$$

where l_{min} is the chromosome length in the most contracted set, L_{min} the total length of this set, l_i and L_i the lengths of the chromosome and the set at any other contraction level. The graphs of change in relative chromosome lengths during spiralisation are segments of hyperbolae (Fig. 3) but there does not seem to be any relationship between chromosome length and spiralisation pattern. The degree of chromosome change is not usually proportional to its length, and sometimes a 'longer' chromosome can become shorter during spiralisation than the 'shorter' one^{5,7}.

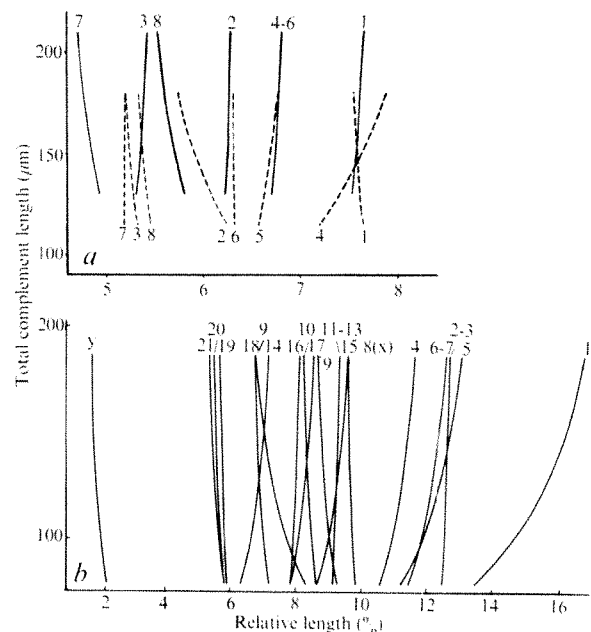


Fig. 3 Dependence of relative lengths of the chromosomes of a, *A. cepa* (solid line), *A. fistulosum* (dotted line); b, *Macaca*, on total complement length.

The correlations between centromeric indices (ratio of the length of the short arm to the whole chromosome length) and the contraction levels are also hyperbolic (Fig. 4). In *Macaca* the differences between the form coefficients of the chromosomes are not so pronounced. This occurs because of an increase in the centromeric indices of subacrocentric chromosomes and a decrease in those of metacentric ones; the latter contradicts the accepted point of view^{2,6}.

Differences in chromosome spiralisations are the result of structural and functional peculiarities of their loci, which can be revealed by the study of the contraction process^{6,7}. The analysis of chromosome parameter dynamics shows that the routine methods of polykaryogram analysis and idiogram construction do not allow the presentation of all karyotype peculiarities because they give only average static patterns from many possible states of the chromosome set.

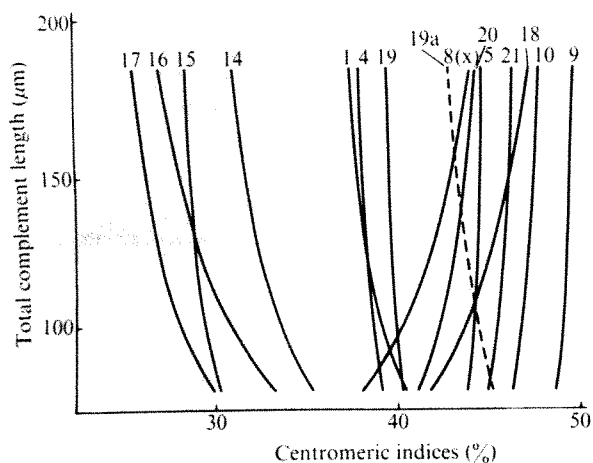


Fig. 4 Dependence of centromeric indices of *Macaca* chromosomes on total complement length.

It also seems that there is sometimes no contraction level which is optimal for the simultaneous morphometric recognition of all the chromosomes. It is possible that some chromosomes which cannot be recognised in less contracted states, differ from one another in the most contracted sets, and vice versa. The idiograms for some chromosomes from the same individual or the same species, constructed for different contraction levels, may differ from one another in relative lengths, form and ability to be identified. From this aspect the optimal representation of karyotype peculiarities is not a static, two-dimensional idiogram, but a three-dimensional idiogram which can show a pattern for every chromosome size and form change during contraction.

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Thymus leukaemia antigen contains β 2-microglobulin

THE major histocompatibility complex (MHC) of the mouse encompasses a number of distinct genetic loci the products of which are expressed on the surface of many types of cell (for review see ref. 1). Some of the gene products may be functionally and structurally related. This is in fact the case for the H-2K and H-2D histocompatibility antigens², which are composed of

two types of polypeptide chain^{3,4}. The larger subunit has a molecular weight of about 50,000 and carries the alloantigenic structures⁵ whereas the smaller subunit has a molecular weight of about 12,000 and seems to be identical to β 2-microglobulin^{3,4}. In view of the involvement of the MHC in several immunological phenomena¹ it is of particular interest that the amino acid sequence of β 2-microglobulin is homologous with those of immunoglobulin G heavy and light chains⁶⁻¹⁰.

It has been postulated that the MHC has arisen by gene duplications¹¹, in which case MHC gene products other than the H-2K and H-2D alloantigens might be expected to share structural features. The thymus leukaemia (TL) antigens in particular seemed likely to repay investigation since these glycoproteins are of a size similar to the H-2 antigens¹²⁻¹⁵. The TL antigens are normally expressed only on thymus cells of some inbred strains of mouse¹⁶. The TL genotype may be universal since leukaemia cells of phenotypically TLA negative mice frequently express the TL antigens¹⁷. We have isolated TL antigens and report that they are composed of two types of subunits, one of which has been identified as β 2-microglobulin.

An antiserum against TL antigens 1 and 3 (ref. 17) was raised by injecting BALB/c mice (H-2^d, TL-2) with A/Sn thymus cells (H-2K^k D^d, TLA-1, 2, 3) at two week intervals for 14 weeks. The antiserum was absorbed with A/Sn spleen and liver cells until its titre tested in a ⁵¹Cr-release cytotoxicity assay¹⁸ on lymph node cells from A/Sn mice was completely abolished. To confirm that all non-TL antibodies had been eliminated, it was demonstrated that no antibodies were bound to C3H thymus cells (H-2^k, TL2) by a sensitive protein A assay¹⁹. The antiserum was, however, highly cytotoxic when examined against A/Sn thymus cells determined both by ⁵¹Cr-release and trypan blue dye exclusion. To obtain highly specific antibodies against β 2-microglobulin a rabbit anti- β 2-microglobulin antiserum was passed over a Sepharose-coupled human β 2-microglobulin column. The eluted antibodies gave a single precipitation line against urinary macromolecules both on immunodiffusion and immunoelectrophoresis³. Cell surface components of A/Sn thymus cells were labelled with ¹²⁵I essentially as described²⁰. Approximately 1×10^8 radioactively-labelled cells were lysed by the addition of NP-40 and the supernatant of the lysate recovered after centrifugation. Aliquots of the supernatant were incubated with TL.1,3 antiserum, normal mouse serum (NMS), or alloantisera against H-2K^k and H-2D^d antigens and precipitated by the subsequent addition of rabbit anti-mouse-IgG serum. The precipitates were collected by centrifugation, solubilised, and subjected to SDS-polyacrylamide gel electrophoresis²¹.

A typical result is shown in Fig. 1. The immune complex formed with the TL.1,3 antiserum and thymus cell antigens contained two types of molecules of molecular weights of about 50,000 and 12,000, respectively. The same antiserum did not form a complex with spleen cell macromolecules. The spleen cell macromolecules contained H-2 antigens both from the K and D ends, however, since alloantisera against these antigens complexed proteins of expected type (Fig. 1b). This result further corroborates that the anti-TL.1,3 serum did not contain detectable antibodies against H-2 antigens.

The large TL antigen polypeptide chain is of a size similar to the H-2 alloantigen-carrying polypeptide chain, in agreement with previous results¹²⁻¹⁵. In addition, the immune complex induced by TL.1,3 antiserum contained a small polypeptide chain of a size similar to β 2-microglobulin. Experiments were therefore designed to investigate the relationship between this polypeptide chain and β 2-microglobulin.

TL antigens were isolated by immunosorbent purification (see legend to Fig. 2). Thymus cell antigens adsorbed to the anti- β 2-microglobulin column contained appreciable amounts of TL antigen since this material could completely abolish the cytotoxic activity of the anti-TLA-1,3 serum. The same material also contained H-2K^k and H-2D^d antigens as determined in a similar way. Spleen cell macromolecules isolated by the same procedure did not impede the cytotoxic activity of the TLA-1,3

antisera but were very efficient in doing so with relevant H-2 alloantisera.

The immunosorbent-purified thymus cell surface antigens contained a small amount of aggregated material in addition to antigens of three distinct molecular weights, that is 50,000, 37,000, and 12,000, determined by gel chromatography in 6 M guanidine hydrochloride (Fig. 2). The figure also demonstrates that immune complexes are formed with the TL α -1,3 antiserum and contain only the 50,000 and 12,000-dalton components. This result also demonstrates that anti- β 2-microglobulin antibodies will bind to TL antigens. Immune complexes formed with H-2K^k and H-2D^d alloantisera contained, however, all three components (not shown). The 37,000-dalton material is indistinguishable from papain-digested H-2 alloantigens (L.R., L.O., and P.A.P., unpublished).

To further prove that the small TL antigen 1,3 polypeptide chain is identical to β 2-microglobulin this polypeptide chain was isolated from TL α -1,3 ¹²⁵I-labelled immune complexes, and mixed with ¹³¹I-labelled β 2-microglobulin obtained from H-2 alloantigens isolated by immune precipitation. The two small polypeptide chains were subjected to polyacrylamide gel electrophoresis at pH 8.9 in 6 M urea. The ¹²⁵I and ¹³¹I labelled components showed indistinguishable electrophoretic mobility and seemed homogeneous. Another part of the material was reduced, alkylated and digested with trypsin. The peptides were separated by two-dimensional electrophoresis and chromatography, radioactive peptides cut out from the paper and analysed for content of ¹²⁵I and ¹³¹I. All radioactive peptides contained both isotopes in a similar ratio.

The present results demonstrate that H-2 and TL-antigens share β 2-microglobulin as a common subunit. The size of the large polypeptide chain carrying the distinctive antigenic determinants is very similar for the H-2 and TL antigens. This TL polypeptide chain may in fact be as similar to H-2 antigens as are H-2K to H-2D antigens (L.O., L.R., and P.A.P., unpublished). Current work in our laboratory to establish the similarity at the level of primary structure between

Fig. 1 SDS-polyacrylamide gel electrophoresis of immune complexes between anti TL-1,3 (○) or NMS (●) and ¹²⁵I lactoperoxidase-labelled A/Sn thymus cell surface antigens (a); and between anti-H-2K^k (●), anti-H-2D^d (○) or anti-TL-1,3 (□) and ¹²⁵I lactoperoxidase labelled A/Sn spleen cell surface antigens (b). The arrows denote the positions of marker IgG heavy and light chains. Experimental details were essentially as described²².

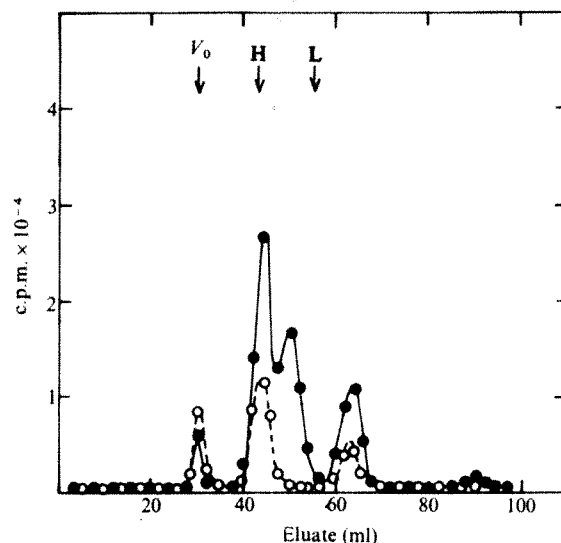
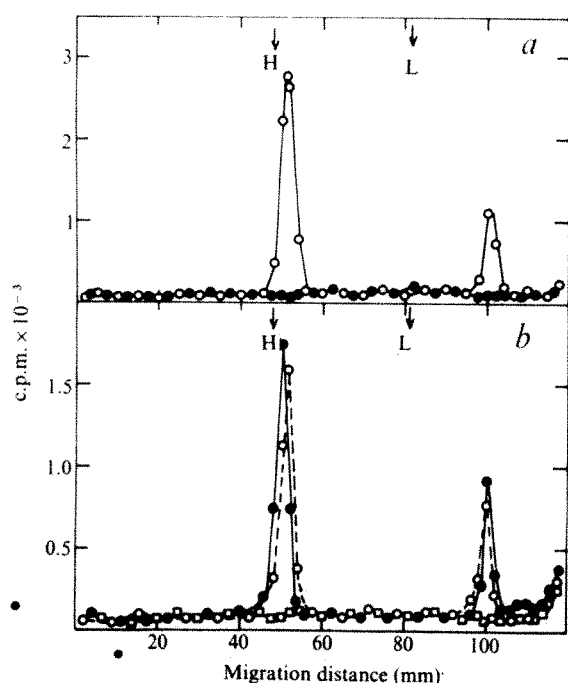


Fig. 2 Gel chromatography of A/Sn thymus membrane antigens on a Sepharose 6 B column equilibrated with 6 M guanidine hydrochloride. Thymus cell membranes were solubilised with 0.5% NP-40 and chromatographed on a column of Sephadex G200. Molecules carrying TL and H-2 antigenic determinants were mainly eluted in a position corresponding to an approximate molecular weight of about 130,000. In the same elution position, the major part of β 2-microglobulin appeared²³. Fractions containing the TL, H-2 and β 2-microglobulin-containing material were pooled, concentrated, passed over a Sepharose-coupled normal rabbit IgG column to remove non-specifically binding material, and subjected to immunosorbent purification on a Sepharose-coupled anti- β 2-microglobulin column²⁴. Material bound to the column was desorbed with 0.05 M sodium citrate buffer, pH 3, containing 0.2 M NaCl. The immunosorbent-isolated proteins were labelled with ¹²⁵I, reduced and alkylated in 6 M guanidine hydrochloride and subjected to gel chromatography in the same solvent (—). Part of the labelled material was complexed with anti-TL-1,3 and precipitated with rabbit anti-mouse IgG. The precipitate was dissolved, reduced and alkylated, and subjected to gel chromatography in 6 M guanidine hydrochloride (— — —). The arrows denote the elution positions of reduced and alkylated IgG light and heavy chains.

the two types of antigens may lend support to the concept that they have had a related genetic evolution.

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After submission of this manuscript it has come to our attention that Fitetta, Uhr, and Boyse have obtained similar results (*J. Immunol.*, in the press).

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Mechanisms for the excitation of 'free nerve endings'

AMONG the sensory nerve endings of mechanoreceptors there are two principal ways in which excitation can occur. In primary endings like the Pacinian corpuscle, the nerve membrane is directly excited by mechanical deformation conveyed by the surrounding non-nervous cells. In secondary endings like those in the ear, modified non-nervous cells are excited by the stimulus. They then excite the sensory nerve membrane either by current flow from cell to cell, or by extracellular field potentials, or finally by some secretory action which is often by way of a chemical synapse but could be of a more diffuse nature¹⁻³. For fine, unmyelinated sensory nerve fibres, however, the dendrites of which branch in tissues to form 'free nerve endings', it is not clear whether excitation is direct or indirect, or whether the endings are primary or secondary. Here I describe a preparation in which the activity of unmyelinated sensory neurones can be recorded simply and where the question is asked: are 'free nerve endings' excited directly by mechanical stimuli or is their excitation dependent on an electrical depolarisation of the cells that they innervate?

This preparation is possible because of some peculiarities in the skin of young amphibian larvae. In *Xenopus laevis*, intracellular recording and stimulation has shown that current can pass from cell to cell in the skin and that the inward-facing plasma membrane of the skin cells is electrically excitable and can propagate an impulse which is Na⁺-dependent, overshoots zero potential and has a duration of about 100 ms (ref 4). This impulse can be evoked by electrical or firm mechanical stimulation of the outside skin surface and, once evoked, propagates in all directions through the skin. The surface of the head is innervated by trigeminal ganglion cells in the brain. The unmyelinated axons (diameters about 1 µm or less) of these cells lose their sheath cells and then branch into the skin, where the slightly vesiculated dendrites run between the cells (Fig. 1A). In the cement gland, the axons do not seem to branch but form an enlarged sensory ending (Fig. 1B and my unpublished work). The impulse activity of these sensory endings can be recorded in the isolated head using suction electrodes placed on the cell bodies in the trigeminal ganglion (Fig. 1C and my unpublished work). Some 67 units have been characterised.

Using this preparation, the receptive field and adequate mechanical stimulation for a particular unit innervating the skin can be defined. The innervated cells can then be induced to fire a skin impulse by stimulation inside or outside the receptive field. Such an overshooting impulse, propagating through the receptive field of the unit, will depolarise the skin cells beyond zero and therefore presumably more than would result from local mechanical stimulation of the cells, which does not evoke an impulse. Consequently, if the 'free nerve endings' are excited by any indirect method dependent on depolarisation of the skin cells one would expect that the skin impulse would excite them to fire impulses. Figure 1C shows that the skin impulse causes a large potential change at the recording electrode but no impulses are evoked in the sensory neurones. This applies to neurones innervating the head skin and cement gland. This result was confirmed by the fact that skin impulses evoked by electrical stimulation did not lead to muscular responses in isolated head preparations whereas stroking with a

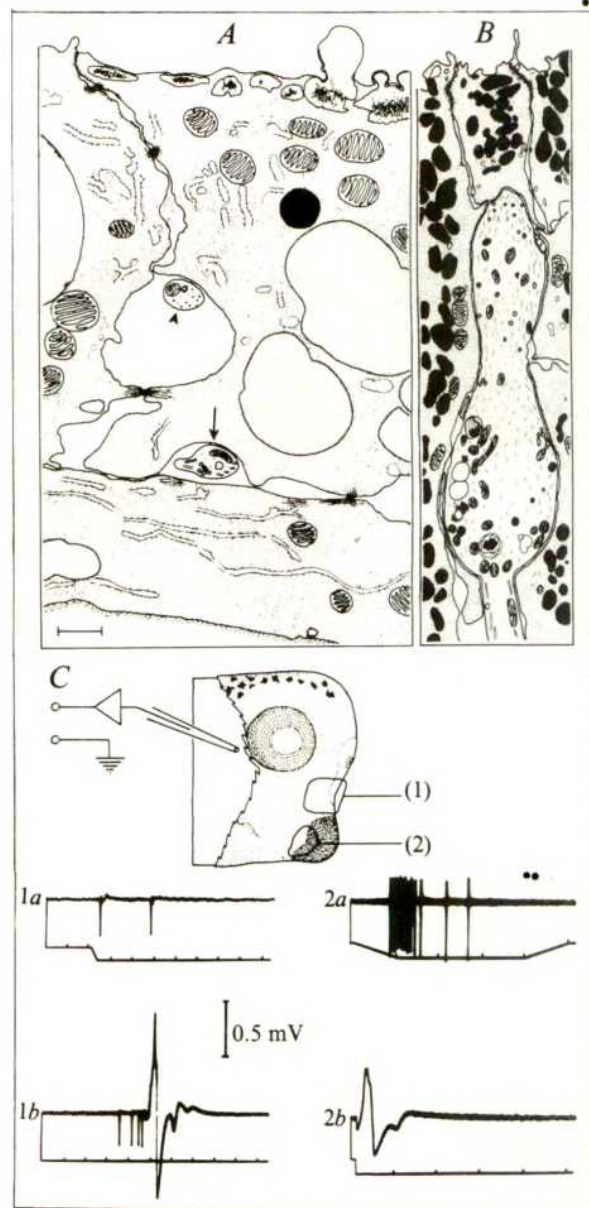


Fig. 1 A and B, Diagrams from electron micrographs to illustrate the two main types of neuronal process found in the skin (A) and cement gland (B). The skin surface is at the top. The sensory dendrites have no sheathing cells but form unencapsulated 'free nerve endings' often closely applied to the cells that they innervate but with no signs of specialised close membrane junctions. The endings contain microtubules and microfilaments (very dense in cement gland endings), clear and dense cored vesicles (20 to 100 nm in diameter), narrow elongated mitochondria, occasional vacuoles and dense bodies (probably lysosomes). In the skin (A) larger sensory processes (arrow) can be seen under the outer layer of cells (with mucus vesicles at their outer surface, black pigment granules and large stippled yolk platelets) while smaller processes (arrowhead) occur between the outer cells. In the cement gland (B) sensory axons form bulbous endings near the secretory surface after running between the elongated gland cells containing numerous black mucus vesicles. There is little extracellular space between mucus cells and the 'free nerve endings'. The 1 µm scale line is at the inner skin surface. C, Diagram to show the isolated head preparation of a young *Xenopus* tadpole. After peeling back the skin behind the eye, sensory activity is recorded in frog Ringer with a glass suction electrode (tip opening 20 µm) on the trigeminal ganglion. The receptive fields of two such neurones are shown with their responses beneath. 1, A unit innervating the skin and responding to a poke in its receptive field with two impulses (1a). A firm stroke evoked four impulses and a skin impulse but no unit impulses during or after the skin impulse (1b). 2, A unit responding to pokes on the cement gland (2a) but giving no response during a skin impulse evoked by a poke to the eye (2b). In each trace the upper beam is the suction electrode record and the lower beam indicates movements of the hair used to deliver pokes plus time marks every 100 ms.

hair to excite the sensory nerves did. This is not the result expected if the skin impulse excites the sensory nerve terminals.

These results indicate that the sensory nerves innervating the head surface of young *Xenopus* (stages 33 to 39) tadpoles⁵ with 'free nerve endings' are not excited by large depolarisations of the skin cells that they innervate. It is therefore very unlikely that the sensory nerve endings are in electrical continuity with the skin cells or that they are excited by secretions liberated from skin cells when they are depolarised by stimulation. It is most likely that the 'free nerve endings' are excited by the direct action of mechanical stimuli in deforming the plasma membrane of the sensory endings. This conclusion could be invalid if skin cells next to sensory nerve processes lost their electrical connections with their neighbours and were not invaded by the skin impulse. In this case many skin cells would be without impulses as the innervation is quite dense (my unpublished work). Though many hundreds of skin cells have been recorded, cells without impulses have not been found in this period of development. Finally, it is possible that mechanical stimulation of the skin could cause secretion without any depolarisation of the skin cells stimulated. If this were the case my conclusions could be wrong.

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Adrenergic receptors in adipose tissue and their relation to adrenergic innervation

THE vascular responses in adipose tissue seem to depend on whether noradrenaline (NA) is released from the sympathetic nerve terminal system or whether it is reaching the receptors by means of the vasculature. Thus electric stimulation of adrenergic nerves to adipose tissue invariably causes α -receptor-mediated vasoconstriction, whereas infusion of NA intravascularly may induce β -receptor-mediated vasodilatation¹⁻⁴. Likewise intravenous tyramine in monkeys causes vasoconstriction in adipose tissue whereas intravenous NA induces vasodilatation⁵. Since tyramine acts by releasing NA from the sympathetic nerve terminal system, these observations also indicate that NA produces qualitatively different effects depending on how it is delivered to the vascular adrenergic receptors. Our results indicate that the vascular adrenergic α -receptors only are located close to the adrenergic nerve terminal system, whereas the vascular β -receptors may have a different distribution, being farther away from the adrenergic nerve terminals. Consequently, the α -receptors may be affected primarily by NA released from the nerve terminals, whereas the β -receptors are primarily stimulated by circulating catecholamines.

We determined, experimentally, changes in sensitivity to NA in canine subcutaneous adipose tissue following chronic denervation. Blockade of the neuronal uptake by denervation should cause supersensitivity, only at those receptor sites which are in close contact with the adrenergic nerve terminal system^{6,7}. The nerve supplying the inguinal subcutaneous adipose tissue on one side was surgically cut in dogs under Nembutal anaesthesia; the other side was sham operated. Seven to eleven days following denervation the dogs were anaesthetised with Nembutal (20 mg per kg body weight) and the subcutaneous adipose tissues in the inguinal region on both sides were isolated

from skin and underlying fascia⁸. The blood flow was measured by arterial drop recorders. The vein was cannulated with plastic tubing to take blood samples for analysis of glycerol⁹. The nerve was transected on the innervated side. Blood pressure was monitored from a carotid artery and blood pressure and blood flows were recorded continuously on a Grass polygraph. The vasodilatory effect of NA was determined after α -blockade with Hydergin (Sandoz) 120-150 μ g intra-arterially. Lipolytic activity was calculated from the net venous outflow of glycerol. Drugs were administered by close arterial injections.

It was found that chronic denervation caused an increased sensitivity of the adipose tissue to NA as far as lipolysis and vasoconstriction were concerned. The potentiation of lipolysis by denervation was of the same order of magnitude both before and after Hydergin administration. This strongly indicates that blockade of neuronal uptake of NA by Hydergin is of minor importance in adipose tissue (compare ref. 10). Vasodilatation was of the same magnitude in the chronically denervated adipose tissue as in the control tissue (Tables 1 and 2).

Chronic denervation did not change the sensitivity of the tissue to isoprenaline (Tables 1 and 2). This indicates that there was no postjunctional supersensitivity. Thus there was no evidence of superinduction of the lipase following chronic denervation as has been found for *n*-acetyltransferase in the pineal organ of the rat¹¹. Increased lipolysis on administration of NA following chronic surgical denervation may be characterised as a prejunctional supersensitivity, probably due to elimination of the neuronal uptake mechanism. This may also be the explanation for the enhanced vasoconstrictor response. An alternative explanation may be that there is a postjunctional nonspecific supersensitivity of smooth muscles to agonist drugs after denervation. This is not likely, however, since there was no change in the vasoconstrictor response to angiotensin (50-300 ng) or metoxamine (2-6 μ g).

The distance between the nerve terminals and the smooth muscles of the vascular bed is an important factor in determining the role of neuronal uptake in termination of the response^{6,7}. With a large neuromuscular gap the neuronal uptake of the transmitter is of minor importance, whereas the reverse is true when there is a small neuromuscular gap. Our experiments indicate, therefore, that the β -receptors on the adipocytes as well as the vascular α -receptors are located close to the adrenergic nerve terminal system. On the other hand, the vascular β -receptors seem to be unrelated to the adrenergic nerve terminal system.

The finding that intravascular administration of a low concentration of NA induces vasodilatation, indicates that the vascular β -receptors are easily accessible to NA diffusing from the intimal side towards the media rather than from the adventitial side where the adrenergic nerve terminals are located. In

Table 1 Lipolytic responses to intra-arterial injections of isoprenaline (Iso) and noradrenaline (NA) without and during α -receptor-blockade [NA (α -block)] in 12 dogs

Drug	Lipolysis Dose (mole)	Glycerol release (μ mol per 100g)
Iso (n = 6)	4×10^{-11} - 1×10^{-10}	C: 2.6 ± 2.1 D: 1.5 ± 1.1 N.S.
Iso (n = 7)	2×10^{-10} - 1×10^{-9}	C: 28 ± 16 D: 26 ± 17 N.S.
NA (α -block) (n = 6)	4×10^{-10} - 1×10^{-9}	C: 5.1 ± 4.6 D: 11 ± 7.7 P < 0.02
NA (α -block) (n = 8)	2×10^{-9} - 1×10^{-8}	C: 18 ± 14 D: 30 ± 16 P < 0.002
NA (n = 8)	4×10^{-10} - 1×10^{-9}	C: 7.0 ± 4.2 D: 24 ± 15 P < 0.01
NA (n = 6)	2×10^{-9} - 1×10^{-8}	C: 41 ± 16 D: 79 ± 28 P < 0.01

Lipolysis is calculated as the net release of glycerol after each injection. The figures given are the mean \pm s.d. C indicates control side, D the denervated side. Statistical hypotheses were tested with Student's *t* test for paired observations.

Table 2 Changes in vascular resistance after intra-arterial injections of isoprenaline and noradrenaline

Drug	Vascular resistance Dose (mol)	% Decrease in PRU
Iso (n = 9)	2×10^{-11} – 1×10^{-10}	C: 39 ± 13 D: 41 ± 15 N.S.
Iso (n = 7)	2×10^{-10} – 1×10^{-9}	C: 69 ± 13 D: 66 ± 10 N.S.
NA (α -block) (n = 6)	2×10^{-10} – 4×10^{-10}	C: 32 ± 17 D: 32 ± 12 N.S.
NA (α -block) (n = 11)	1×10^{-9} – 10^{-8}	C: 64 ± 12 D: 63 ± 9 N.S.
NA (n = 7)	2×10^{-10} – 4×10^{-10}	% Increase in PRU C: 59 ± 33 D: 92 ± 77 N.S.*
NA (n = 9)	1×10^{-9} – 4×10^{-9}	C: 128 ± 78 D: 210 ± 114 $P < 0.002$

The vascular resistance was calculated as the blood pressure over blood flow and was expressed in peripheral resistance units (PRU). A decrease in PRU indicates vasodilatation, an increase vasoconstriction. The percentage change is calculated from the maximal responses after each injection.

* The increase in PRU was greater on the denervated side in six out of seven experiments.

the latter case the vascular α -receptors seem to be in a better position to be combined with NA. Thus the vascular β -receptors in adipose tissue seem to be functionally related to circulating rather than to neurally released NA. From the functional point of view, therefore, they may be classified as humoral β -receptors whereas the vascular α -receptors as well as the β -receptors on the adipocytes may be classified as innervated receptors.

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Thyrotrophin-releasing hormone and shaking behaviour in rat

THYROTROPHIN-RELEASING hormone (TRH) produces behavioural effects in experimental animals¹ and may have psychoactive properties in man^{2–5}. Winokur and Utiger⁶ and Brownstein *et al.*⁷ have described the distribution of TRH in rat brain, suggesting that TRH has a modifying role in synaptic functions in addition to its effect on the pituitary. The behavioural effects of endogenous TRH release in the brain are not known; however, Prange *et al.*⁸ noted that the systemic administration of TRH to pentobarbital-anaesthetised rats resulted in lacrimation, paw tremor and a peculiar shaking movement of the head and trunk. These behavioural effects were also obtained in partially anaesthetised rats following intracisternal injection of 10 μ g TRH per animal. The TRH-induced shaking was particularly interesting because we have observed this behaviour as a characteristic sign of morphine abstinence in the anaesthetised rat⁹. Here, we have studied the central sites of TRH-induced shaking to determine if these sites parallel the endogenous distribution of TRH in the rat

brain and also to determine if these sites correspond to brain areas where morphine withdrawal shakes are obtained.

Male Sprague-Dawley rats (180–240 g) were anaesthetised with a 2.5% sodium pentobarbital solution, 50 mg kg⁻¹ intraperitoneally, and 30 min later mounted on a small animal stereotaxic instrument (David Kopf, Tujunga, California). TRH (Calbiochem, La Jolla, California) was dissolved in 0.9% saline and injected intracerebrally using the micro-injection technique described by Hedge *et al.*¹⁰. All injections were made bilaterally at a volume of 0.5 μ l per hemisphere. The anatomical sites of injection were verified by histological examination of brains after injection of 0.5% methylene blue dye into control animals (see stereotaxic coordinates in Table 1). After intracerebral injections, animals were taken from the stereotaxic instrument and placed in a supine position. Shaking, defined as any quick rotational movement of the head, shoulders, or body was counted for 15 min.

Table 1 Regional sensitivity of the rat brain to the effects of TRH, 0.5 μ g per animal intracerebrally, on shaking behaviour (n = 8 for each brain area)

Target area in brain	Stereotaxic coordinates (mm) [†]			No. of shakes (mean \pm s.e.)
	Ant.	Lat.	Vert.	
Saline in PAG4*	-0.5	0.50	5.5	1.2 \pm 0.7
Substantia nigra	1.75	2.25	6.5	4.1 \pm 2.4
Fasciculus retroflexus	3.5	0.75	5.5	1.4 \pm 0.9
Medial hypothalamus	5.0	0.50	7.5	22.4 \pm 6.7 [‡]
Medial thalamus	5.5	0.50	4.5	34.2 \pm 9.6 [‡]
Thalamic reticular nucleus	5.6	2.0	5.5	4.0 \pm 1.7
Medial preoptic area	7.25	0.50	7.0	14.9 \pm 3.9 [‡]
Basal ganglia	9.0	2.3	5.5	9.7 \pm 0.4
Frontal cortex	10.0	3.0	2.0	0

*Data for TRH injections into the periaqueductal-fourth ventricle (PAG4) area are shown in Fig. 1; animals in all other brain areas received TRH.

[†]The coordinates, based on the atlas of König and Klippel²¹, refer to anterior (Ant.) from lambda, lateral (Lat.) to the midline and vertical (Vert.) from the dura. The upper incisor bar was placed at 2.4 mm below the interaural line.

[‡] $P < 0.01$ against saline, Mann-Whitney U test according to Siegel²².

Injection of TRH into sensitive brain areas produced shaking, lacrimation, paw tremor and intense shivering. After a challenge dose of 0.5 μ g TRH per rat, we found that brain regions exhibiting a significant degree of shaking were located in the periaqueductal-fourth ventricular spaces (PAG4), the medial thalamus, the medial hypothalamus and the medial preoptic area; the substantia nigra, areas around the fasciculus retroflexus, reticular nucleus of the thalamus, basal ganglia and the frontal cortex were relatively inactive (Table 1). The dose-effect curve for TRH-induced shaking in the PAG4 area is shown in Fig. 1. Using six or more shakes as a quantitative index of response, the median effective dose of TRH for eliciting shaking in the PAG4 area was calculated, according to the method of Litchfield and Wilcoxon¹¹, as 0.12 (0.06–0.23) μ g per rat.

These results indicate that the neuroanatomical sites of TRH-stimulated shaking parallel the endogenous distribution of TRH in the rat brain^{6,7}. The apparent localisation of TRH-sensitive areas in the medial mesodiencephalon and medulla may provide clues to the endogenous functions of TRH. The medial mesodiencephalon and the medulla are generally considered to be important integration areas for the control of shivering^{12–16}. The behavioural syndrome of intense muscular activity and vasoconstriction obtained with intracerebral TRH injections suggest that heat gain mechanisms were activated by TRH. It would be of interest to determine if TRH acts, in its postulated modifying role in synaptic functions, as a

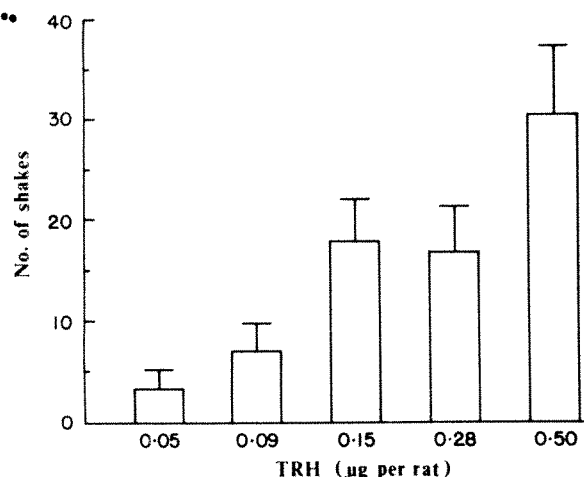


Fig. 1 The dose-effect curve for TRH-induced shaking in the periaqueductal-fourth ventricle area. $n = 8$ for each dose level.

chemical mediator of behavioural and hormonal mechanisms which enable the organism to adapt to cold.

Shaking and vasoconstriction are characteristic abstinence signs which appear in anaesthetised, morphine-dependent rats after the administration of naloxone, an opiate antagonist⁹. The brain areas where naloxone precipitates withdrawal shaking in morphine-dependent organisms¹⁷⁻¹⁹ parallel the sites of TRH-stimulated shaking and the endogenous sites of distribution of TRH (refs 6 and 7). The relationship of some opiate abstinence signs to TRH-linked mechanisms in the brain merits further investigation, since studies by George²⁰ have shown that morphine can act on discrete areas of the hypothalamus to affect the release of thyrotrophin. It should also be noted that the shaking behaviour observed with TRH, and with naloxone in the morphine-dependent animal, exhibits a high degree of chemical specificity, in that various substances such as noradrenaline HCl (0.2–5 µg), dopamine HCl (5 µg), L-isoproterenol HCl (5 µg), carbamylcholine chloride (0.2–1 µg), ecothiophate iodide (2 µg), pilocarpine (3 µg), 5-hydroxytryptamine-creatinine sulphate complex (0.02–10 µg), prostaglandin E₁ (5 µg), L-glutamic acid (0.5 µg), L-proline (0.5 µg), L-histidine (0.5 µg), saturated KCl, 2-deoxy-D-glucose (10 µg), 0.2 N NaOH, and 0.2 N HCl, when injected into the PAG4 area under the same experimental conditions as the TRH injections, did not provoke shaking (E.W., and S.S., unpublished).

In summary, we suggest that TRH activates pathways in the brain which lead to heat gain and that the central pharmacological effects of TRH resemble some abstinence signs seen during morphine withdrawal.

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Photopigment conversions expressed in pupil mechanism of blowfly visual sense cells

VISUAL sense cells of flies are depolarised at illumination¹⁻³. This event triggers the pupil mechanism: pigment granules dispersed throughout the cytoplasm migrate towards the rhabdomere, that is, the light-guiding rod-like part of the sense cell which contains the visual pigment molecules⁴⁻⁸. The amount of pigment granules accumulated at the boundary of the rhabdomere controls, through absorption and scattering, the light flux in the photoreceptor⁴.

We have already investigated⁷ the pupil mechanism of the blowfly *Calliphora erythrocephala* by measuring the transmission of the photoreceptors and found that the absorbance of the pigment granules is extreme in the blue and minimal in the yellow. We concluded that, for the photochemical cycle of fly rhodopsin, the visual pigment conversions can be appropriately described by P495 \rightleftharpoons M580; in other words, that

the photoproduct metarhodopsin M580, absorbing maximally in the yellow, can be regenerated into rhodopsin P495 absorbing blue-green, both photochemically (\rightleftharpoons) and by a dark process (\leftarrow), (refs 7,9,10). We also proposed that, in view of the blue-peaking pupil absorbance spectrum, the essential function of the pupil is a spectral filter action, protecting rhodopsin from excessive photoconversion⁷. Here we present new evidence to support this view, obtained from investigations on the reopening of the pupil during dark adaptation as a function of the previous light adaptation which caused pupil closure.

The pupil was investigated experimentally using the sensitive technique of measuring the antidromic transmission¹¹ of the photoreceptors. The tip of a quartz rod was introduced into the back of the head of the blowfly and a steady test light was applied by way of this rod and the antidromic transmission, that is, the light fraction transmitted by the photoreceptors and leaving the eye through the facet lenses, was detected^{6,7,11,12}. The test wavelength of 508 nm was chosen at about the isobestic point of the visual pigment⁷, thus avoiding transmission changes resulting from visual pigment conversions; the test light intensity was below the threshold of pupil activation. Activation of the pupil mechanism was induced by irradiating the eye of the fly in the normal, orthodromic¹¹ way for 3 s. Within this light adaptation time the pupil always reached a new equilibrium.

First, intense red illumination, 603 nm, was given to create an almost pure rhodopsin content. After a dark adaptation time t_d , monochromatic illumination of wavelength λ_s closed the pupil, observable as a decrease in transmission. During the subsequent darkness the opening time course of the pupil was determined by measuring the course of the transmission increase. The half time t_k of this opening process (inset of Fig. 1a) is of the order of a few seconds if the preceding illumination

intensity is moderate; but t_k increases monotonically with light intensity, the effect being strongest with blue illumination. The phenomenon of maintained pupil closure notwithstanding renewed darkness we have called the 'glueing effect', because the pupil granules of the visual sense cells seem to remain more or less 'glued' to the rhabdomere.

Figure 1a presents t_k as a function of wavelength λ_s for irradiation of extremely high intensity, showing that the 'glueing effect' is strongest at about 465 nm. These results were obtained with a fixed dark time, $t_d = 2$ min, whereas those in Fig. 1b were obtained with varying t_d and fixed wavelengths $\lambda_s = 584$ nm and 457 nm, respectively. Figure 1b shows that, while the yellow light, at 584 nm, hardly affects the reopening speed of the pupil even after the longest t_d chosen, the same t_d when followed by blue illumination at 457 nm, induces a very strong 'glueing' effect. In other words, especially blue light has a tremendous capacity for slowing down dark adaptation.

How are the photopigment conversions tied up with the migration of the pupil granules? The simple supposition that metarhodopsin production closes the pupil and that the decay of metarhodopsin during the dark reopens it cannot cover the facts: rhodopsin dark regeneration is far too slow for that, the process having a half time of about 30 min⁷. Furthermore, the spectrum of Fig. 1a has a maximum at 465 nm, distinctly to the left of the rhodopsin peak, which is situated at about 495 nm. Starting as we do with pure rhodopsin an intense blue stimulus induces excessive conversion of rhodopsin into metarhodopsin and we have hypothesised that the lack of the opposite conversion has been responsible for the 'glueing' at the cessation

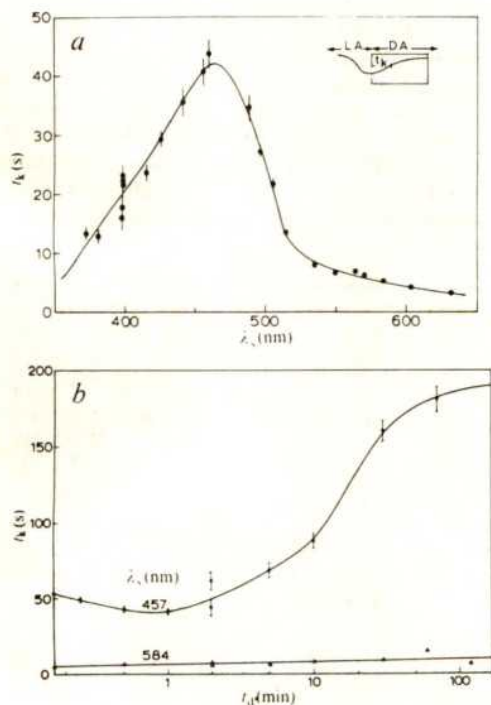


Fig. 1 Reopening of the pupil of the fly after intense illumination. The pigment granules inside the photoreceptor cells of the fly migrate at light adaptation (LA) towards the rhabdomere. The lightflux propagated in and along the rhabdomere decreases as a result of an accumulation of absorbing and scattering pigment granules near the rhabdomere boundary⁴. This transmission decrease (inset) is followed by an increase in transmission during subsequent dark adaptation (DA), as a result of the redistribution of the pigment granules throughout the visual cell soma. The pigment granules thus fulfil a pupil function. The half time t_k of the reopening of the pupil during darkness is determined as a function of the wavelength λ_s of the preceding illumination; this irradiation in turn followed a fixed time of $t_d = 2$ min darkness during which all visual molecules were in the rhodopsin state (a). The same procedure, but now with a varying darkness time t_d and fixed wavelengths, 584 nm and 457 nm, respectively, yielded the t_k values presented in (b). It proves that blue light induces a heavily 'glueing' pupil.

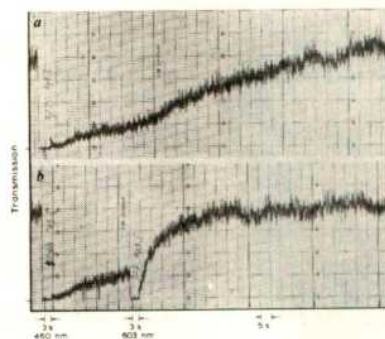


Fig. 2 Experimental transmission curves of the pupil of the blowfly. After a dark adaptation time of 4 min, closure of the open pupil is quickly induced by a 3 s intense blue illumination, 460 nm (during which the photomultiplier is protected from overstimulation). In the following darkness a very slow reopening of the pupil results (a). Repetition of the experiment, but now with an intense red illumination at 603 nm during the dark recovery shows an initial reclosure of the pupil; however, in renewed darkness the pupil shows a markedly enhanced opening speed. Clearly, red irradiation counteracts the 'glueing' effect of the blue light.

of the blue stimulus. Since metarhodopsin is converted into rhodopsin by long wavelength light the hypothesis predicts that red light will reverse the pupil.

To verify this prediction (Fig. 2) we again created pure rhodopsin photochemically, subsequently giving a dark adaptation time $t_d = 4$ min. Having closed the pupil with an intense 3-s blue illumination, $\lambda_s = 460$ nm, another period in the dark results in only a very slow reopening of the pupil (Fig. 2a). An intense red illumination at 603 nm, applied at the onset of this recovery phase promptly closed the partly reopened pupil again, but at the termination of this period the pupil quickly and completely reopened (Fig. 2b). Thus the rhodopsin production caused by the red light might well be responsible for cutting short the 'glueing' effect. We therefore conclude that the two opposite visual pigment conversions induce antagonistic actions in the pupil of the fly.

Antagonistic actions of visual pigment conversions observed in receptor potential measurements in barnacle¹³ and *Limulus*¹⁴ photoreceptor cells must surely be closely related to our results. Antagonistic action of the visual pigment conversions $P \rightleftharpoons M$ and $M \rightleftharpoons P$ on the photoreceptor membrane may occur quite generally¹⁴, although the actual absorption spectra can differ widely: in the barnacle P532 and M495, in *Limulus* ultraviolet-receptor P360 and M480 and in the fly P495 and M580. The two opposite photopigment conversions also influence the early receptor potential in both barnacle¹⁵ and fruitfly¹⁰ photoreceptor cells.

These analogies further support the view that the receptor potential and the force pulling the pigment granules through the cytoplasm of the receptor cell are intimately connected^{5,6,12}. A useful working hypothesis for pigment migration is an electrophoresis model^{5,12} in which it is assumed that on illumination the photopigment conversions locally alter the properties of the receptor cell membrane of the rhabdomere, so that both a changed membrane potential and an intracellular force field are created. In the latter field the presumably charged pigment granules will then migrate towards the rhabdomere. In renewed darkness, recovery of the receptor cell is expressed by a simultaneous dying away of both the receptor potential and the force field, the latter being recognisable as an opening pupil. Brownian motion will disperse the granules until a random distribution is reached.

Encouraging evidence for this model can be drawn from measurements on the receptor potential of the blowfly¹⁶ following the same experimental procedure as that giving rise to Fig. 2a. The time course of the receptor potential was strikingly similar to that of the pupil action.

The benefit derived from the strong bathochromic shift,

occurring at conversion of fly P495 into M580, may now be seen. Both red and yellow screening pigments in the eye of the fly leave the stray red light virtually undisturbed⁷. The metarhodopsin, absorbing yellow-red, is therefore converted photochemically into rhodopsin by this stray light, thus favouring dark adaptation (compare Fig. 2).

We have found that dark adaptation is slowed down considerably by blue irradiation. Note that the blowfly pupil absorbance spectrum peaks also in the blue⁷. We therefore conclude that the pupil function is not merely the control of light flux quantity but also the control of its spectral composition in such a way that the cell is not severely desensitised after intense illumination (as for instance by the sun and the sky).

In conclusion, flies seem to have carefully evolved the properties of their photostable pigments in combination with those of their visual pigment. The essential advantage of the pupil to the investigator is that it allows optical probing of visual sense cells and so can reveal further details of both primary visual processes and the generated intracellular force field.

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Opsonin and leukophilic γ globulin in chronically splenectomised rats with and without heterotropic autotransplanted splenic tissue

CHRONICALLY splenectomised animals reflect depressed macrophage activity because the serum immunoglobulins that stimulate them, such as opsonins^{1–6} and leukophilic γ globulins^{7–8}, are depressed. After splenectomy in animals receiving heterotropic autotransplants of splenic tissue, the tissue fragments (after initial necrosis) are reorganised into structures that are microscopically indistinguishable from the original spleen^{9–11}. These reorganised explants also perform erythrophagocytosis and have been known to protect against *Bartonella muris* infections that are usually fatal to splenectomised rats. Furthermore, the cells from these explants can exhibit both humoral and cell-mediated immune reactions¹². I report here that similar explants of autologous splenic tissue can protect against the deficient opsonin and leukophilic γ globulin activity observed in splenectomised rats.

Twenty-four recently weaned Fischer 344 female rats (40–50 g; Charles River Breeding Laboratories) were separated into three groups of eight and one of the following procedures was performed on each animal of a group: (1) sham splenectomy, (2) splenectomy or (3) splenectomy followed by transplantation

of a third of the autologous spleen. Using aseptic techniques, surgical splenectomy (under sodium phenobarbital anaesthesia, 5 mg per 100 g body weight) was performed through a left lateral subcostal skin and peritoneal incision and the spleen was removed. Each rat of one group received approximately one-third of the autologous spleen, which was placed in a subcutaneous pocket made in the left lateral thoraco-axillary region using the blunt end of forceps, and the skin was sutured with autoclips. Three months later preparations of autologous polymorphonuclear (PMN) leukocytes and serum from each animal of each group were used for phagocytosis studies according to the method of Rogers and Melley¹³ as modified by Fidalgo and Najjar⁷.

By intracardiac puncture, blood was obtained from each animal for separation of opsonin and leukophilic γ globulin. Usually about 3 ml of heparinised blood was obtained for cell preparations and 3 ml of clotted blood was used for serum. The buffy coat was removed using siliconised Pasteur pipettes, washed three times (300g) and resuspended using Krebs–Ringer phosphate glucose buffer, pH 7.4 (KRPB). Incubation mixtures consisted of autologous PMN leukocytes and serum (or γ G-globulin) with *Diplococcus pneumoniae* type III (pneumococcus) as target particles. Serum-opsonin activity was evaluated using complement-inactivated (56 °C, 2 h) serum or γ G-globulin (separated by Fahey's method)¹⁴ incubated with PMN leukocytes and unopsonised pneumococcus. Serum leukophilic γ globulin activity was determined using complement-inactivated sera and γ G-globulin preparations (that were 'deopsonised' by exhaustive adsorption with pneumococci). Autologous PMN leukocytes and opsonised pneumococci (incubated with serum at 37 °C for 1 h) were used in these incubation mixtures.

Phagocytosis was studied in silicone-coated glass-stoppered tubes at 37 °C, rotated slowly in a circular rotator. The components of the reaction mixture, added within 1 min, consisted of (1) 0.1 ml leukocytes (2×10^7 – 4×10^7 PMN cells) in KRPB; (2) 0.1 ml of KRPB or 0.1 ml of autologous serum that was either complement-inactivated (for evaluation of opsonins) or in addition, opsonin-depleted (for studies evaluating leuko-

Table 1 Opsonin activity in sera from splenectomised and splenectomised-autotransplanted rats

Rat no.	Cells and KRPB (0.1 ml)	Phagocytosis rates*	
		Complement-inactivated serum (0.1 ml)	Complement-inactivated γ G-globulin (2 mg)
1	10	22	26
2	11	24	24
3	12	20	27
4 Control	15	Mean = 11.0	23
5	14	28	28
6	10	20	31
7	12	24	23
8	14	30	29
9	10	15	16
10	11	16	17
11	13	12	16
12 Splenectomy	12	Mean = 12.8	16
13	12	15	15
14	15	14	16
15	14	14	17
16	15	14	13
17	10	26	28
18	12	28	27
19 Splenectomy-	14	31	30
20 splenic	15	Mean = 13.1	34
21 autotransplant	13	30	31
22	16	33	34
23	14	33	33
24	11	29	34

Each value represents the mean of triplicate samplings rounded to the nearest number.

*Unopsonised *D. pneumoniae* used in all incubation mixtures.

Table 2 Leukokinin activity in sera from splenectomised and splenectomised-autotransplanted rats

Rat no.	Cells and KRP (0.1 ml)	Phagocytosis rates*	
		Cells and complement-inactivated deopsonised serum (0.1 ml)	Cells and complement-inactivated and deopsonised γ G-globulin (2 mg)
1	16	37	35
2	18	37	38
3	20	38	40
4 Control	21	Mean = 18.5	Mean = 35.1
5	19	31	37
6	17	32	35
7	20	35	34
8	17	35	36
9	16	20	16
10	19	19	21
11	22	20	17
12 Splenectomy	15	Mean = 18.1	Mean = 21.1
13	17	25	23
14	21	21	20
15	17	22	17
16	18	20	21
17	16	40	36
18	22	35	37
19 Splenectomy-splenic	18	39	39
20 autotransplant	19	Mean = 18.4	Mean = 35.8
21	21	32	34
22	17	34	35
23	16	36	32
24	18	38	35

Each value represents mean of triplicate samplings rounded to the nearest number.

*Opsonised *D. pneumonae* used in cell incubation mixtures.

phobic γ globulin activity), or 0.1 ml of KRP containing 2 mg of autologous γ G-globulin; and (3) 0.1 ml of KRP containing approximately 4×10^7 opsonised bacteria (leukophilic γ globulin activity) or unopsonised pneumococci (opsonin activity). After 30 min of incubation, a sample mixture was removed on a loop of diameter 4 mm and mixed with a similar sample of bovine serum albumin (60 mg ml⁻¹ KRP). The mixture was smeared on coverslips, air dried and stained with Wright's stain. Phagocytosis index was expressed as the number of PMN containing one or more pneumococci per 100 cells counted, 600 PMN cells counted independently each by two observers. All assays were made in triplicate.

Tables 1 and 2 show that sera or γ G-globulin from normal and splenectomised-autotransplanted rats (unlike sera or γ G-globulin from splenectomised rats) had normal opsonin and leukophilic γ globulin activity. The opsonin studies (Table 1) revealed that PMN leukocytes from control animals, in the presence of sera or γ G-globulin, were stimulated into phagocytosis of unopsonised pneumococci from a basal rate of 11.0% (KRP) to 25.0% (serum) and 25.4% (γ G-globulin). Enhanced phagocytosis (from a basal rate of 13.1%) was also observed when autologous serum (28.0%) or γ G-globulin (31.1%) from splenectomised-autotransplanted rats was included in the incubation mixture. Sera or γ G-globulin from chronically splenectomised rats did not enhance phagocytosis (basal rate: 12.8% with (KRP); with serum 14.5%; γ G-globulin: 15.5%). Evaluation of leukophilic γ G-globulin activity from sera of these animals revealed similar findings (Table 2). Autologous PMN leukocytes in the presence of opsonin-depleted sera or γ G-globulin from control animals exhibited a stimulation of phagocytosis of opsonised pneumococci from a basal rate of 18.5% to 35.1% with serum and to 36.9% with γ G-globulin. Rates were similar when serum or γ G-globulin from rats with splenic autotransplants were used (KRP 18.4%; serum 35.8%; γ G-globulin 35.3%). The sera or γ G-globulin from splenectomised rats failed to enhance phagocytosis (KRP 18.1%; serum 21.1%; γ G-

globulin 19.5%). Results were similar when these experiments were repeated.

To evaluate the time necessary for the depletion of serum opsonin and leukophilic γ globulin in rats after splenectomy, 60 recently weaned Fischer 344 females were separated into three groups of 20 and each animal of a group was subjected to either (1) sham-splenectomy, (2) splenectomy or (3) splenectomy followed by autotransplantation of splenic tissue. Opsonin and leukophilic γ globulin activity was evaluated on four rats of each group after 3, 6, 9, 12 and 15 weeks. The sera from sham-splenectomised rats and those receiving autotransplants of splenic tissue exhibited enhanced rates of phagocytosis of pneumococci by PMN leukocytes at all time intervals. The sera from splenectomised animals exhibited optimal opsonin and leukophilic γ globulin activity for 9 weeks followed by a precipitous absence in these activities at 12 weeks and thereafter. When these experiments were repeated the results were similar after 20 and 30 weeks. The serum of these splenectomised animals did not stimulate PMN leukocytosis of pneumococci.

These studies revealed that the depression of opsonin and leukophilic γ globulin activity observed in the sera of chronically splenectomised animals (when compared with the sham-splenectomised controls) could be avoided after ectopic autotransplantation of splenic tissue. The depressed opsonin activity was evidenced by inability of complement-inactivated sera or γ G-globulin to enhance phagocytosis of unopsonised pneumococci by autologous PMN leukocytes. The lack of leukophilic γ globulin activity in sera from splenectomised rats was observed when autologous PMN leukocytes were incubated in the presence of opsonised pneumococci and complement-inactivated sera from which the opsonins had been removed. Optimal opsonin and leukophilic γ globulin activity was exhibited by the sera (or γ G-globulins) from the control and splenectomised autotransplanted rats. The depletion of opsonin and leukophilic γ globulin activity between 9 and 12 weeks following splenectomy tended to parallel the half life of γ G-globulin¹⁵⁻¹⁶. Recent studies performed here suggest that leukophilic γ globulin activity is represented by the γ G₁-globulin moiety of the rat γ G-globulin pool.

The spleen has been known to be essential for the recruitment of lymphocytes to antigenic site for sensitisation^{17,18} as well as production of antibody towards particulate antigens¹⁹⁻²¹. Splenectomised and hereditary asplenic mice reflect defective production of antibodies of the γ -M and γ -G₂ class which may paralleled the lack of thymus-bone marrow synergism that has been observed in these animals²²⁻²⁴. These asplenic mice also exhibit defective production of interferon following infection with Newcastle disease virus that is corrected after neonatal transplantation of splenic cells²⁵. Depressed opsonin and leukophilic γ globulin have also been observed in children who have undergone splenectomy²⁶⁻²⁹. These children exhibit defective phagocytosis and they are also confronted with a high incidence of fulminant and fatal sepsis³⁰⁻³⁴. Whether subcutaneous implantation of splenic tissue (not reflecting hypersplenism) in children undergoing splenectomy would correct these defects remains to be seen.

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Reactivity to tumour-associated antigens detected in mice undergoing liver regeneration

IN addition to the well-known, individually unique tumour-specific antigens in chemically induced neoplasms and the common ones in neoplasms induced by the same virus, there is evidence that neoplastic cells have antigens which are present in certain normal, syngeneic cells, but which may still be reacted against immunologically¹. Most notable has been the demonstration that many tumours share antigens with normal embryonic cells². Immunity to these antigens can be detected in various ways. It has been shown, for example, that lymphocytes from multiparous mice are cytotoxic to cultivated neoplastic but not to normal adult cells³⁻⁵ and that serum from pregnant mice can inhibit (block) this cytotoxic effect⁴⁻⁷.

We describe here data indicating that lymph node cells (LNC) from mice which have undergone partial hepatectomy, followed by liver regeneration, are cytotoxic to cultivated neoplastic cells but not to normal cells and that serum from the hepatectomised mice can block the cytotoxic effect of LNC from multiparous or hepatectomised mice. Our findings suggest that

a normal tissue repair process, involving rapid cell division, may lead to a detectable immunological reaction against antigens present on neoplastic cells.

Partial hepatectomy was performed on BALB/c and C3H mice following established procedures, removing 65-75% of the hepatic parenchyma⁸; the reason for including C3H mice was that these survived hepatectomy better than the BALB/c mice. Littermates of the hepatectomised mice underwent sham-hepatectomy, consisting of anaesthesia, opening of the peritoneal cavity and manipulation of the liver. At various times after hepatectomy, mice were bled from the retro-orbital sinus and their sera stored for later testing of blocking activity. Animals were killed at 7-10-d intervals and tested for LNC-mediated reactivity to cultivated neoplastic cells.

One transplantable BALB/c methylcholanthrene-induced sarcoma line, No. 1315, propagated in culture for approximately one month; a BALB/c Moloney sarcoma virus-transformed 3T3 line (3T3-MSV); BALB/c fibroblasts from the skin of newborn mice, and non-transformed BALB/c 3T3 cells were used as targets in microcytotoxicity assays; this cell material has been described before⁶.

Microcytotoxicity tests for LNC-mediated reactivity and blocking serum activity were performed using Falcon Microtest II plates as previously described⁶; doses of 150,000 and 75,000 (occasionally 25,000) LNC per well and a serum dilution (in tests for blocking activity) of 1:10 were regularly used. The sera were allowed to remain with LNC and target cells for the 30-36-h duration of the experiments.

Absorptions to remove blocking serum activity were performed, as previously described⁶, by incubating 1:5 diluted sera from hepatectomised or control mice for 45 min at 37°C with equal volumes of cell suspensions from regenerating (or normal, control) liver or tumour 1315; the cell suspensions had been prepared mechanically and contained approximately 10⁶ cells per ml. After centrifugation, the absorbed sera were tested for blocking activity.

Table 1 shows that LNC from hepatectomised mice, compared with LNC from sham-operated controls, were cytotoxic to the two tumour lines used as targets. They had no consistent cytotoxic effect against the untransformed, control cells of the same strain as the tumour cells; in some experiments a cytotoxic effect was seen against the control cells which was generally weaker than that detected against the tumour cells, while in other experiments a stimulation of the control cells (negative

Table 1 Cytotoxic effect of lymph node cells from hepatectomised mice on cultivated neoplastic cells

Experiment no.	Days between hepatectomy and test*	No. of effector cells per well × 10 ³	No. 1315 tumour cells	% Reduction of target cells per well		
				3T3-MSV tumour cells	BALB/c fibroblasts	3T3
1	10	150	28.8†	20.6†	15.9	14.0
		75	37.0‡			
	34	150	33.9‡	15.8	7.0	11.5
2	8	150	35.2§			
		75	30.7‡	5.4	33.0‡	0
	18	150	9.1	-26.9	-7.8	-97.3§
3	22	150	28.4†	22.6†	25.0†	23.7†
		75	43.2§	40.2‡	-30.5‡	26.7‡
	10	150	42.5§	13.4	-18.4	1.3
4	22	150	28.1†	32.0‡	-9.1	-6.7
		75	32.1	38.9§	-3.6	9.2
	81	150	21.8		-10.0	
5	11	150	29.8†			
		75	13.4		-21.9†	
	13	150	-33.5		-21.9†	
6	15	150	3.4			
		75	26.7†		3.3	
	17	150	29.1‡		-14.1	
7	25	150	46.6§		NT	
		75	48.0§		-3.9	
	10	150	17.7†			

* C3H mice were used as lymph node cell donors except in part of experiment 1 (34-d mouse), part of experiment 3 (81-d mouse), and experiment 5, when BALB/c mice were used.

Probabilities that differences between experimental groups (hepatectomised) and controls (sham-operated) are due to chance indicated: † $P < 0.05$, ‡ $P < 0.01$, § $P < 0.00$. NT, not tested.

Table 2 Blocking of the cytotoxic effect of lymph node cells from multiparous or hepatectomised mice on tumour cells by serum from hepatectomised mice

Experiment no.	Days between hepatectomy and bleeding*	Blocking effect of 1:10 diluted serum tested with			
		1315 % Reduction control serum†	% Blocking by hepatect. serum§	3T3-MSV % Reduction control serum†	% blocking by hepatect. serum¶
1	7	19.1‡	10.5	21.7	> 100
	17		52.4		> 100
	21		> 100		> 100
	33		NT		> 100
	66		< -100		> 100
2	7	48.7‡	53.8		
	7-10		67.8		
	21		80.1		
3	80	32.5‡	-7.8		
	11		> 100		
	13		62.2		
4	15	17.1‡	> 100		
	7		-0.6		
	7		27.7		
	19		38.8		
5	23		-86.5		
6	8-15	30.8§	43.8		
7	10-15	17.7§	72.3		
8	8-22 absorbed control liver	21.0‡	62.5		
	absorbed regenerating liver		-20.7		
	absorbed 1315 tumour		< -100		
	8-22 absorbed control liver	46.1‡	96.0		
	absorbed regenerating liver		-41.6		
	absorbed 1315 tumour		14.2		
	unabsorbed		> 100		

* Sera from C3H mice were used except in experiments 6-8 when sera from BALB/c mice were used.

† Serum from matched sham-operated mice used as controls. Percentage reduction of target cell numbers per well with LNC from multiparous or hepatectomised compared with age matched virgin BALB/c females indicated.

‡ LNC from multiparous mice were used for the experimental group.

§ LNC from hepatectomised mice were used for the experimental group.

¶ Blocking serum activity calculated from % reduction with LNC from multiparous or hepatectomised BALB/c in the presence of experimental group (hepatectomised) compared with control (sham-operated) serum. NT, not tested.

cytotoxicity) was observed. Lymph node cell-mediated tumour cell cytotoxicity was seen as early as 8 d and as late as 81 d after hepatectomy. There was no discernable difference whether hepatectomised BALB/c or C3H mice were studied.

Preliminary data indicate that the cytotoxic effect of spleen cells from hepatectomised donors on tumour cells is not removable by passing them through immunoglobulin columns, or by removal of macrophages, while reactivity decreases after incubation of the spleen cells with anti-theta serum and complement. The nature of the effector cells needs to be studied further, however.

Table 2 shows that sera from the hepatectomised mice could abrogate the cytotoxic effect of LNC from multiparous and hepatectomised mice, as compared with sera from sham-operated controls; this was seen whether sera from C3H or BALB/c mice were studied. The blocking effect was detected as a greater number of surviving target cells in the presence of immune lymphocytes and blocking serum than in the presence of immune lymphocytes and control serum; a few of the blocking sera slightly depressed the number of remaining target cells in the presence of control lymphocytes, but most of them did not. Often the blocking serum effect was seen as soon as 7 d after hepatectomy and it remained 2-4 weeks. Two sera taken more than 2 months and one taken 23 d after hepatectomy lacked blocking activity; two of these sera increased the LNC effect.

The blocking activity of sera from hepatectomised mice could be removed by absorption with regenerating liver or No. 1315 tumour cells, while absorption with liver from sham-operated control mice did not remove it.

The specificity demonstrated by the absorption experiments makes it more likely that an immune reaction to some antigens present on tumour cells and regenerating liver cells is responsible for our findings, rather than a more nonspecific destruction of tumour cells, mediated, for example, by activated macrophages. The latter possibility cannot, however, be definitely ruled out and would, if correct, be consistent with the demonstration that

neoplastic cells are more sensitive to a cytotoxic effect of non-specifically activated macrophages than are their non-neoplastic counterparts¹⁰.

One interpretation of the present data, which is worthy of consideration as an impetus for further work, is that undifferentiated cells present in normal, regenerating liver possess antigens which can immunise their host in such a way that both an LNC-mediated cytotoxic response and a blocking serum activity can be measured against neoplastic target cells. One may speculate that this could be because some antigens are shared by tumour cells, embryonic cells and certain rapidly multiplying normal cells. It is interesting in this context that foetal enzymes have been detected in regenerating liver¹¹ and that α -foetoprotein has been demonstrated in the sera of animals undergoing liver regeneration¹². It would then also be tempting to speculate that an immune response to antigens present on certain, perhaps less differentiated, normal cells may provide an "immunostimulation"¹³ of their growth *in vivo*, for example during liver regeneration, and that the blocking serum activity detected *in vitro* may play a role in such a stimulation *in vivo*; there is, however, no direct evidence either for or against this idea. Since a high level of cell multiplication occurs even without partial hepatectomy, for example in bone marrow, intestines and skin, the lymphoid cell-mediated cytotoxicity and blocking serum activity we have detected must be assumed to be the outcome of a change of a normally balanced level, perhaps representing some type of surveillance mechanism.

Finally, we wish to point out that our data suggest one possible explanation why lymphoid cells can be sometimes more cytotoxic to a variety of tumour cells than to normal cells. The cytotoxic effect may have resulted from some repair process, involving stimulation of normal cell growth and immunisation against some antigens present on rapidly dividing cells or, alternatively, the possible activation of macrophages. Our findings do not, of course, argue against the specificity of immune reactions to tissue type specific or individually unique

tumour antigens detected on human and animal neoplastic cells¹.

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Effects of free fatty acid and enzyme release in experimental glucose on myocardial infarction

In physiological concentrations, circulating long chain free fatty acids (FFA) can inhibit the myocardial oxidation of glucose and are the preferred fuel of the heart in the fasted state^{1,2}. In sufficiently high concentrations, fatty acids as the only fuel can have toxic effects on the normal perfused rat heart³. The mechanisms involved in fatty acid toxicity may include the intracellular accumulation of products of fatty acid metabolism such as acyl CoA (ref. 4), which inhibits the transfer of ATP from sites of synthesis in the mitochondria to sites of utilisation in the cytoplasm. Thus, enhanced rates of FFA metabolism could be expected to aggravate myocardial infarction, in which there is a loss of ATP in the poorly perfused tissue predomi-

antly supplied by the occluded artery⁵. As an intracellular ATP deficiency is postulated to be a factor promoting release of enzymes⁶, increased provision of FFA to the heart should accelerate enzyme release from infarcting heart tissue. Conversely, if the rate of accumulation of FFA breakdown products were decreased by the additional provision of glucose or glucose plus insulin to promote intracellular esterification of FFA, then the rate of enzyme release should be decreased. Increased provision of glucose could also result in increased production of anaerobic ATP which could be of importance in promoting the survival of the oxygen-limited myocardium⁷.

We tested these hypotheses in isolated rat hearts, perfused by the left atrium and performing external work⁸, which were subject to ligation of the left coronary artery, thereby reducing coronary blood flow to about 30–46% of its normal flow⁹. Hearts were perfused for 1 h with media containing glucose, or albumin-bound palmitate, or palmitate together with glucose and glucagon-low insulin (Table 1). The rate of release of lactate dehydrogenase¹⁰ was taken as an index of the severity of ischaemic cell damage. Release of lactate dehydrogenase from ligated hearts was four to eight times greater with palmitate-albumin as substrate than with glucose-albumin. Increasing the molar ratio of palmitate-albumin from 1:1 to 5:1 doubled the rate of release of lactate dehydrogenase, in accordance with the effects of higher FFA-albumin molar ratios which accelerate FFA uptake by tissues¹¹. The addition of glucose and insulin to media containing palmitate-albumin decreased the rate of release of lactate dehydrogenase by about two-thirds. Patterns of release of creatine phosphokinase and aspartate aminotransferase were similar to those of lactate dehydrogenase (groups 5 and 6, Table 1); several enzymes are released in parallel in other experimental situations in perfused heart studies¹². Serious arrhythmias, such as ventricular fibrillation, were not found in our experiments although expected at high FFA-albumin molar ratios¹³.

The magnitude of enzyme release from the infarcting heart has been related to the ultimate extent of heart cell necrosis (infarct size) in dogs¹⁴ and in man¹⁵. Our results show that the nature of the substrate reaching the infarcting myocardium can greatly influence the magnitude of enzyme release and can therefore be expected also to influence the ultimate severity and extent of cell necrosis. We provide the first direct evidence for the hypothesis that increased provision of glucose is beneficial and increased provision of FFA is harmful to the infarcting tissue^{16,17} by altering the

Table 1 Effects of various substrates and of insulin on the rate of release of lactate dehydrogenase (LDH) into the medium of isolated perfused working rat hearts with coronary artery ligation

Substrate(s) and insulin concentration	Albumin concentration	FFA-albumin molar ratio†	LDH release U g ⁻¹ h ⁻¹	P‡
1. Glucose 11 mM	0.44 mM*	—	2.6 ± 0.15 (5)	
2. Palmitate 0.5 mM	0.44 mM	1.1	10.7 ± 1.17 (8)	<0.0002 (against 1)
3. Palmitate 1.5 mM	0.44 mM	3.4	19.5 ± 2.51 (4)	<0.004 (against 2)
4. Glucose 11 mM	0.1 mM	—	2.6 ± 0.27 (5)	
5. Palmitate 0.5 mM	0.1 mM	5.0	21.3 ± 1.77 (8)	<0.0002 (against 2)
6. Palmitate 0.5 mM + glucose 11 mM	0.1 mM	5.0	15.3 ± 0.57 (5)	<0.025 (against 5)
7. Palmitate 0.5 mM + insulin 2 mU ml ⁻¹	0.1 mM	5.0	12.9 ± 2.44 (6)	<0.01 (against 5)
8. Palmitate 0.5 mM + glucose 11 mM + insulin 2 mU ml ⁻¹	0.1 mM	5.0	8.1 ± 1.11 (6)	<0.0001 (against 5) <0.004 (against 6)

Enzyme activities measured at 25°C on perfusion fluid sampled from recirculation system. Mean values ± s.e.m. (number of hearts). Substrate concentration = initial value at start of perfusion.

* 3 g 100 ml⁻¹.

† For importance of FFA-albumin molar ratio, see ref. 11.

‡ P values calculated from Student's *t* test for two-tailed values.

Rates of release of creatine phosphokinase were (U g⁻¹ h⁻¹): group 4, 1.2 ± 0.5; group 5, 5.2 ± 1.10 (*P* < 0.01). Rates of release of aspartate aminotransferase were (U g⁻¹ h⁻¹): group 4, 0.8 ± 0.07; group 5, 3.0 ± 0.4 (*P* < 0.001).

extent of metabolic damage. In addition, the decreased rates of enzyme release after the addition of insulin to perfusates containing FFA-albumin in the absence of glucose suggest that insulin has a direct protective action on the ischaemic cell, possibly by inhibition of lysosomal activity¹⁸. Further work is required to determine whether the substrates reaching the heart *in situ* could be changed sufficiently to influence the outcome of myocardial infarction in man.

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Active sodium transport by mammalian urinary bladder

ALTHOUGH sodium reabsorption by the urinary bladder is important to urine formation in amphibia^{1–3}, there have been few studies of ion movements^{3–7} across the mammalian bladder. If the mammalian bladder were an inert sac exhibiting only passive ion fluxes, as generally assumed, ion concentration gradients established between urine and plasma by the kidney would tend to dissipate in the bladder. We now report findings that may resolve this problem: mammalian bladder possesses, in addition to an exceptionally high electrical resistance, an aldosterone-stimulated Na⁺ reabsorptive mechanism.

Sheets of rabbit urinary bladder were mounted *in vitro* between temperature-controlled (37° C) Plexiglass chambers with 2 cm² exposed. The bathing solution was 110 mM NaCl, 7 mM KCl, 25 mM NaHCO₃, 2 mM CaCl₂, 2 mM MgSO₄, 1.2 mM NaH₂PO₄, and 10 mM glucose, gassed with 5% CO₂–95% O₂ (pH 7.4). Three technical problems were encountered. (1) Contraction of the thick muscle layers of intact bladder made membrane characteristics unstable. We therefore dissected away most of the muscle before mounting. The smooth muscle contractant acetylcholine did not affect transepithelial resistance (*R*) or capacitance (*C*) of the resulting epithelial sheet. (2) Ideally, measurements of membrane properties should be normalised to membrane area. The area of epithelium actually exposed in a 2 cm² hole is unknown and variable, because bladder folding varies with stretch. Measurements were therefore normalised to effective epithelial capacitance (*C*, determined from the membrane time constant τ as τ/R)⁸, as a measure of actual epithelial area in cm². For single-cell membranes 1 cm² area corresponds to a capacitance of about 1 μ F (ref. 9). For

bladder, *C* corresponding to 2 cm² chamber area varied with stretch from 1.9 to 5.4 μ F. Such normalised measurements (for example, $\Omega \mu$ F, μ A μ F⁻¹) showed much less variability among preparations than did measurements normalised to chamber area. (3) Edge damage (disruption of cells by the chamber edges, producing high-conductance shunts¹⁰) was avoided by using silicone lubricant (Dow-Corning high vacuum grease) and a flanged chamber design with 20 mounting pins. With these techniques paracellular shunt resistance for rabbit urinary bladder exceeds 300,000 $\Omega \mu$ F, so that edge conductance is undetectable⁸.

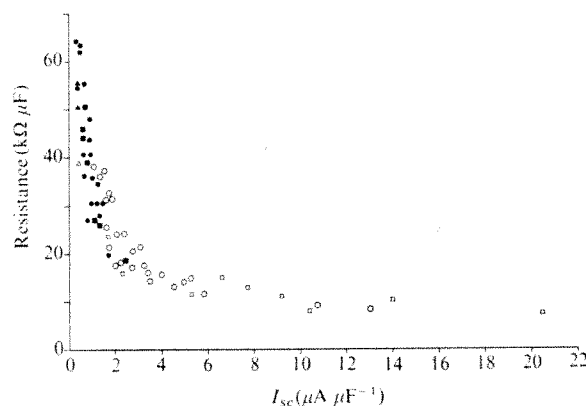


Fig. 1 Relationship between transepithelial resistance (ordinate) and short-circuit current (abscissa) of rabbit urinary bladder, both normalised to effective transepithelial capacitance. Note that *R* and *I*_{sc} are inversely correlated. Animals on normal (□), low-Na⁺ (○), or high-Na⁺ (■) diets; bladder exposed *in vitro* to 10⁻⁴ M amiloride (●) or 10⁻³ M 2,4,6-triaminopyrimidine (▲) (ref. 15; S. A. L. and J. H. Moreno, unpublished observation); Na⁺ in bathing solutions replaced with choline (△).

Electrical parameters of bladders separating identical solutions varied considerably among animals. The spontaneous potential (*V*) was 24–70 mV (mean \pm s.e.m., 45 ± 2 mV (*n* = 34)), serosal surface (blood side) positive to mucosal surface (urine side). Short-circuit current (*I*_{sc}) was 0.5–20.5 μ A μ F⁻¹ (3.0 ± 0.4 (*n* = 34)), the sign corresponding to a net mucosa-to-serosa flux of cations. *R* was 9,000–65,000 $\Omega \mu$ F ($23,000 \pm 1,750$ (*n* = 34)). Spontaneous variations in *I*_{sc} and *R* showed a striking inverse correlation (Fig. 1). *V*, *I*_{sc}, and *R* were approximately constant for 9 h after dissection and then began to decrease.

Three procedures had opposite effects on *I*_{sc} and *R*. (1) Stepwise replacement of Na⁺ with choline in the mucosal solution (but not in the serosal solution) reversibly reduced *I*_{sc} towards zero and increased *R*. (2) When added to the mucosal solution at 10⁻⁴ M, the natriuretic drug amiloride¹¹ reversibly reduced *I*_{sc} by $85 \pm 9\%$ (*n* = 9) and increased *R* by $93 \pm 45\%$ (*n* = 9), with a half-time of 3 s. (3) At 10⁻⁶ M the anti-natriuretic hormone aldosterone¹² increased *I*_{sc} by $88 \pm 23\%$ (*n* = 6) and reduced *R* by $35 \pm 6\%$ (*n* = 6), after a lag of 45 min.

These three effects on *I*_{sc} and *R* suggest that the actively transported ion is Na⁺. ²²Na and ³⁶Cl flux measurements confirmed this conclusion. Mucosa-to-serosa flux (*J*_{ms}) of ²²Na varied linearly with *I*_{sc} (Fig. 2), while serosa-to-mucosa flux (*J*_{sm}) of ²²Na was very small and independent of *I*_{sc}. The ratio (*J*_{ms} - *J*_{sm})/*I*_{sc} was 1.03 ± 0.08 (*n* = 28). In contrast, both *J*_{ms} and *J*_{sm} of ³⁶Cl were very small, approximately equal, and independent of *I*_{sc}. (*J*_{ms} - *J*_{sm})/*I*_{sc} for ³⁶Cl was only -0.08 ± 0.09 (*n* = 9). Thus, Na⁺ accounts for all the *I*_{sc}, and Cl⁻ accounts for none of it. On open circuit the ratio *J*_{ms}/*J*_{sm} was 7.53 ± 1.45 (*n* = 10), and the net flux (*J*_{ms} - *J*_{sm}) was 1.23 ± 0.14 (*n* = 10) μ A μ F⁻¹ for Na⁺ but only 0.15 ± 0.06 (*n* = 9) μ A μ F⁻¹ for Cl⁻. Thus the main counter-ion for Na⁺ on open circuit is not Cl⁻, but might be either HCO₃⁻ or a serosa-to-mucosa flux of K⁺ and/or H⁺.

The inverse relationship between *I*_{sc} and *R* follows the same curve (Fig. 1), whether *I*_{sc} and *R* are manipulated by choline-

for Na^+ replacement, amiloride, aldosterone or variation among animals. Evidently, rabbits vary individually in a system that includes a Na^+ pump and a Na^+ conductance pathway which are somehow stimulated by aldosterone and inhibited by amiloride. Rabbit urinary bladder epithelium consists of three cell layers in series. Microelectrode techniques⁸ have shown that the open-circuit voltage and transepithelial resistance reside entirely in the cell layer nearest the mucosal solution, and that changes in resistance of the cell membrane nearest the mucosal solution are entirely responsible for the observed variation in R with I_{sc} . Thus, the Na^+ conductance pathway resides in this membrane.

To test for a physiological role of bladder Na^+ transport, we placed rabbits on either a high or low- Na^+ diet for 7–14 d and monitored urine $(\text{K}^+)/(\text{Na}^+)$ ratios as an index of Na^+ retention. At the end of this period the bladder transport properties were studied *in vitro*. The high- Na^+ diet was found to decrease I_{sc} and urine $(\text{K}^+)/(\text{Na}^+)$ (compared with values for animals on a normal diet). The low- Na^+ diet had the opposite effect and probably led to high plasma aldosterone levels, as reflected in the high urine $(\text{K}^+)/(\text{Na}^+)$ ratio¹³. These results suggest that bladder V , I_{sc} , and R respond to physiological release of aldosterone as well as to *in vitro* doses.

Under conditions of restricted Na^+ intake (low urine (Na^+)), a steep concentration gradient exists for passive diffusion of Na^+ from plasma to bladder lumen. The function of the Na^+ pump in bladder is presumably to maintain the urine (Na^+)

properties to membrane capacitance may also be relevant to other epithelia.

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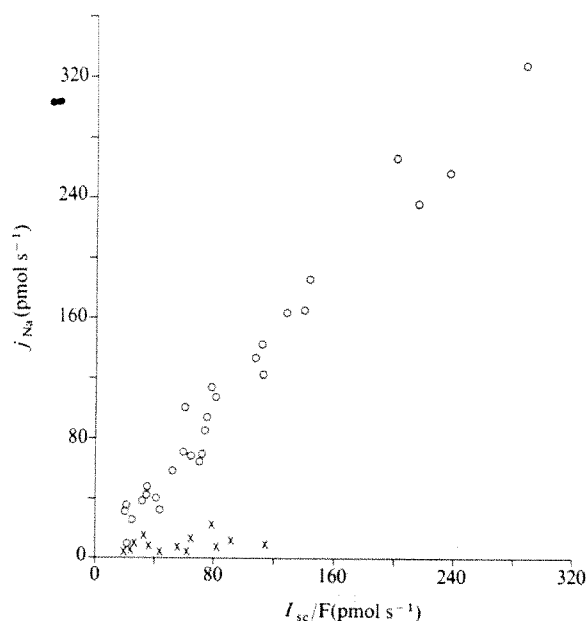


Fig. 2 Relationship between one-way tracer flux of ^{22}Na (ordinate) and total net flux of ions calculated from I_{sc} as I_{sc}/F (abscissa). ○, Mucosa-to-serosa flux J_{ms} ; ×, serosa-to-mucosa flux J_{sm} . Note that variation in J_{ms} but not in J_{sm} is closely correlated with variation in I_{sc} .

constant by actively transporting Na^+ from lumen to plasma, thus preventing dissipation of the Na^+ gradient. In addition, most of the ion permeability of the bladder is associated with the aldosterone-stimulated, amiloride-inhibited pathway, through which Na^+ can pass from urine to blood but perhaps not in the reverse direction. When this pathway is inhibited by amiloride or a high Na^+ diet, bladder resistance is very high ($>45,000 \Omega \mu\text{F}$). Thus, the permeability properties of the bladder minimise plasma-to-urine leakage of ions. Because of its high transepithelial resistance, unmeasurably high paracellular resistance, apparent functional equivalence to a single cell layer, and presence of one cell type in this layer, mammalian urinary bladder provides a convenient experimental system for understanding tight epithelia¹⁴. The techniques we developed for minimising edge damage and normalising membrane pro-

Xeroderma pigmentosum variants have decreased repair of ultraviolet-damaged DNA

XERODERMA pigmentosum (XP) is an inherited human disease characterised by the development of pigmentation abnormalities and numerous malignancies on sun-exposed areas¹. Fibroblasts from typical patients with XP are defective in the repair of ultraviolet-produced damage of their DNA: These cells show (with respect to normal fibroblasts) decreased amounts of ultraviolet-induced unscheduled DNA synthesis¹⁻³, less ultraviolet-induced uptake of bromodeoxyuridine into parental DNA (refs 2 and 4), and greater sensitivity to ultraviolet irradiation in terms of colony forming ability⁵⁻⁷. The relationship of the DNA repair defect(s) to the clinical manifestations of XP, however, has been obscured by the finding of a group of patients, designated XP 'variants'⁸, who have the clinical manifestations of the disease^{1,8}, but whose cells lack the repair defects (Table 1). Using a sensitive host-cell reactivation technique, I have found that fibroblasts from patients belonging to all five known variant kindreds have defects in the repair of DNA damaged by ultraviolet irradiation.

The host-cell reactivation technique used to estimate repair, presumably DNA repair, has been described previously^{9,10}. Briefly, non-irradiated and ultraviolet-irradiated suspensions of adenovirus 2 (a nuclear-replicating, double-stranded DNA virus) were assayed for their ability to form plaques on monolayers of human fibroblasts. Non-irradiated viruses or viruses repaired by the fibroblasts form plaques on such monolayers. This technique has previously been used to quantitate defective DNA repair in typical XP fibroblasts which had between 3% and 50% of normal host-cell reactivation⁹. Using this technique, I found that fibroblasts from the first reported variant, XP4BE, showed approximately 70% of the repair capacity of normal fibroblast strains⁹. This study was inconclusive as only one variant strain was then available for study. To determine whether the defective repair in this variant was characteristic of all variant strains, four strains from the other four known variant kindreds were obtained: all five variant strains (last column, Table 1) have defective repair manifested by similar degrees of decreased host-cell reactivation of ultraviolet-irradiated adenovirus 2.

Figure 1 shows results from two of the five experiments performed to obtain the data in Table 1. Each frame of Fig. 1 presents survival curves of irradiated adenovirus 2 using two

Table 1 Characteristics of the five known XP variant strains

Fibroblast strain	Ultraviolet-induced unscheduled DNA synthesis	Response to ultraviolet irradiation Ultraviolet-induced incorporation of bromodeoxyuridine into parental DNA	Ultraviolet sensitivity of colony forming ability	Reactivation of adenovirus 2 (% normal)
XP4BE	Normal*	Normal*§	Normal†	64
XP13BE	Normal‡	Normal†	Normal†	60
HG859	ND	Normal§	ND	63
XP7TA	Normal	Normal¶	ND	57
XP30RO	Normal	Normal	ND	57

*Ref. 1.

†Ref. 8.

‡K. H. Kraemer and J. H. Robbins (unpublished).

§J. D. Regan and R. B. Setlow (unpublished).

¶H. Slor (unpublished).

||Ref. 13

ND, not done.

The percentage of normal repair was estimated by dividing the dose at which adenovirus survival was 37% (D_{37}) using XP variant fibroblasts as viral hosts (see legend to Fig. 1) by the average D_{37} obtained using normal fibroblasts ($237 \pm 24 \text{ J m}^{-2}$). XP4BE is from Patient 4, NIH XP Series¹, also designated 1162 (refs 9 and 10). Other fibroblast strains from this patient were designated XP16SF (ref. 8), XW (refs 9 and 10), and patient 4 (ref. 3). XP13BE, from patient 13, NIH XP Series, was obtained from the American Type Culture Collection (CRL 1258). XP14SF (ref. 8) is from the same patient. HG859 is a strain taken from a 25-yr-old girl who has eight siblings, four of whom have XP. This strain and information were kindly given by Dr James German, New York Blood Center, New York. XP7TA was prepared by Dr Hanoch Slor, and was obtained through Dr Colin Arlett, University of Sussex. The family of the patient from whom this strain was started has three of seven children with XP (H. Slor, personal communication). XP30RO was grown out in the laboratory of D. Bootsma from a biopsy taken from a 30-yr-old man, the only one involved in his family (E. A. de-Weerd Kastelein, personal communication) by V. Der Kaloustian, Beirut, Lebanon, and provided by Dr Arlett.

normal and three variant fibroblast strains as viral hosts. The variant strains gave rise to steeper viral inactivation curves than did the normal strains, reflecting less repair of the irradiated virus by the variant than by the normal strains. From such slopes the ultraviolet doses required to reduce the virus survival to 37% (D_{37} s) were calculated. By dividing the D_{37} s obtained with each variant strain by the average D_{37} obtained with the normal strains, the relative host-cell reactivation abilities were obtained (Table 1, last column). Previous measurements using the adenovirus host-cell reactivation test to estimate repair levels in ten normal human fibroblast strains (KD, ND and 1119 included) gave an average $D_{37} \pm \text{s.d.}$ of

$222 \pm 20 \text{ J m}^{-2}$ indicating that the normal strains used here are behaving as expected⁹. The average D_{37} obtained here for the XP variant strain XP4BE of 152 J m^{-2} is in good agreement with the value of $153 \pm 15 \text{ J m}^{-2}$ obtained from analysing 20 previous experiments on this strain.

These host-cell reactivation studies show that all the XP variants are unable to repair ultraviolet-irradiated adenovirus as well as normal cells. They do not indicate, however, which repair process is defective. It has been reported^{11,12} that fibroblasts from three of the XP variants have abnormal post-replication repair, but it is not yet known whether such abnormal postreplication repair is the cause of the decreased host-cell reactivation of ultraviolet-irradiated adenovirus 2 in the variants.

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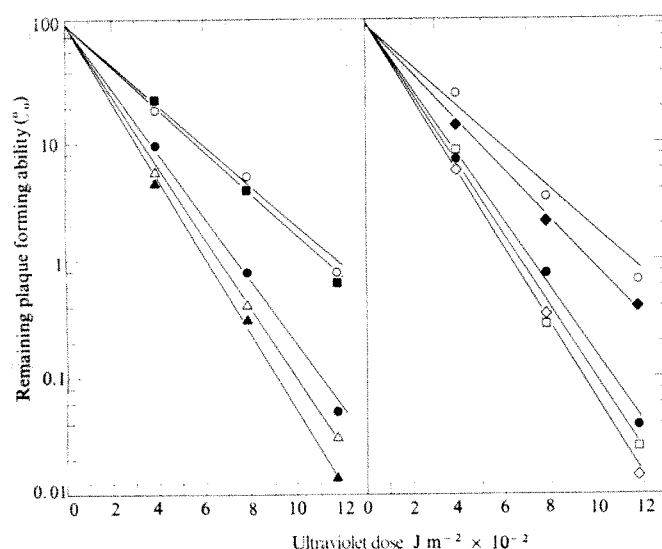


Fig. 1 XP variants' abnormal repair of ultraviolet-irradiated adenovirus 2. Adenovirus 2 was prepared, diluted, irradiated, and plaque on fibroblasts of the various strains as described previously^{9,10}. XP variant strains: ●, XP4BE; ▲, XP7TA; △, XP30RO; ◇, HG859; □, XP13BE. Normal fibroblast strains: ■, 1119; ○, KD; ◆, ND. Average D_{37} is the dose required to reduce virus survival to 37%. Average D_{37} (range), and the number of experiments (n) were as follows. XP4BE: 152 (143-157), $n = 5$; XP7TA: 136 (133-138), $n = 2$; XP30RO: 136 (129-143), $n = 2$; HG859: 149 (137-157), $n = 3$; XP13BE: 143 (139-146), $n = 3$; 1119: 248 (241-254), $n = 2$; KD: 257 (243-275), $n = 3$; ND: 210, $n = 1$; HG800: 212 (207-217), $n = 2$ (data not shown). Normal fibroblast strain HG800 was kindly supplied by Dr James German. The origins of the other normal fibroblast strains have been described^{9,10}.

Poly(A) size class distribution in globin mRNAs as a function of time

STUDIES on the mouse globin mRNAs have shown that specific size classes of poly(A) exist in a population of labelled globin mRNAs (refs 1 and 2). These size classes are present in both α and β globin mRNAs (ref. 1) and the overall size of the poly(A) region in these mRNAs decreases with time¹. As the three discrete size classes previously reported were observed

Table 1 Poly (A) distribution of size classes

Time	Size class	c.p.m.	Specific activity c.p.m. mg ⁻¹	pmol	% pmol	Labelled poly(A) molecules (%)
6 h	Small	602	160	327	79	51
	Intermediate	729	600	53	13	32
	Large	710	600	34	8	17
	Total	2,041	230	414	—	—
10 h	Small	844	435	206	77	62
	Intermediate	819	940	46	16	30
	Large	378	940	14	7	8
	Total	2,031	635	264	—	—
20 h	Small	1,807	1,900	100	78	80
	Intermediate	802	2,100	20	16	15
	Large	451	2,100	8	6	5
	Total	3,060	2,055	128	—	—

Since the amounts of mRNA digested were not equal, the counts from each peak in Fig. 1 were converted to pmol using the specific activity and molecular weight of each size class. Specific activities for small and combined large and intermediate fractions were obtained by Millipore filter fractionation. The percentage labelled poly(A) was calculated from the c.p.m. in each fraction and the molecular weight. This differs from percentage c.p.m. in that the larger size classes will have more counts per molecule.

at one labelling time, we determined whether these same size classes occur at various stages of messenger maturation, as their presence during the overall shortening process could be indicative of a specific degradatory mechanism. The presence of limiting steps in the shortening of the poly(A) region of mouse globin mRNAs is interesting in the light of possible shortening mechanisms and their relationship to poly(A) binding proteins^{3,4} and mRNA stability.

To obtain mRNAs of different age, a ³²P labelling regimen was used which followed the maturation of the mRNA synthesising erythroid precursor cells. When the mRNA was

digested and the poly(A) region analysed the size classes observed previously were present at all times. The amount of the larger size classes, however, decreased at longer labelling times with a corresponding increase in the smallest size class. The change in the distribution of the size classes was calculated to obtain kinetic data for possible models of poly(A) shortening.

Reticulocytosis was induced in Swiss Cox mice as described previously¹. Mouse globin mRNA was labelled *in vivo* by injecting 2 mCi ³²P per mouse at 20, 10 and 6 h before collection of reticulocytes, to insure the same degree of reticulocytosis in all animals at all time points. When an anaemic animal is injected with ³²P, the erythroid precursor cells incorporate the label into RNA; however, only labelled RNA from those cells which can mature between time of labelling and reticulocyte collection will be analysed.

The poly(A) fragments can be categorised into three major size classes based on the 20 h distribution: one migrating slightly heavier than 4S RNA, one migrating intermediate between 4S and 5S and a shoulder migrating heavier than 5S (Fig. 1). These results are consistent with those reported previously for an 18-h labelling period¹. At earlier times the fragments in the shoulder could be tentatively resolved into two or three peaks.

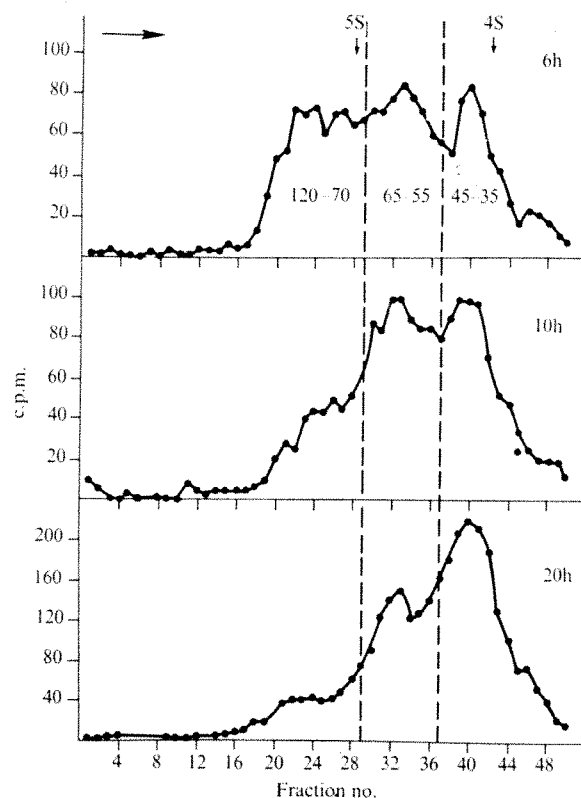


Fig. 1 12% SDS polyacrylamide gels of ³²P poly(A)-containing fragments. Reticulocyte polysomes were collected as described previously⁵. RNA was extracted using the phenol-chloroform-isomyl alcohol technique⁶ and the globin mRNA isolated by two passages through oligo(dT)-cellulose as described previously⁶. The globin mRNAs were digested with RNase A and T₁ and the poly(A) fragments isolated as described previously^{1,7}. The size of the poly(A) regions was determined using electrophoresis. The fragments are divided into three major size classes (dotted lines) corresponding to lengths of 35-45; 55-65; and 70-120 nucleotides.

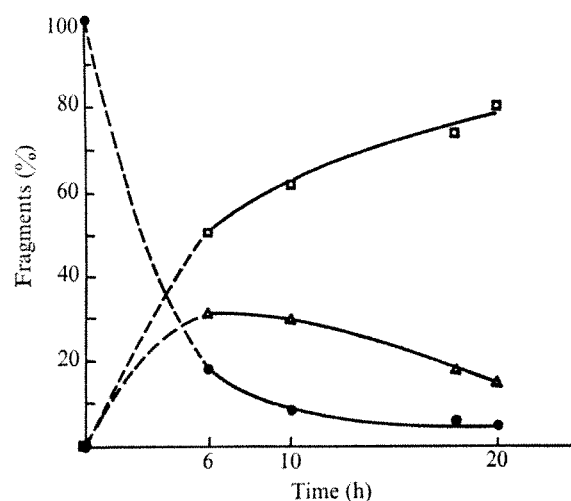


Fig. 2 Plot of percentage poly(A) fragments in each size class present at various times after labelling (last column of Table 1). □, A₃₅₋₄₅; △, A₅₅₋₆₅; ●, A₇₀₋₁₂₀.

For a kinetic analysis of the data it is important to determine the precursor pool. From the specific activity of erythroid precursor cell rRNA and mRNA, it seems that the rate of incorporation is constant for 10 h, indicating that the pool is constant for this time and satisfies the condition for a con-

tinuous labelling experiment. The rate of label incorporated into the reticulocyte RNA is also constant during the times assayed, as the specific activities of reticulocyte mRNA are comparable to those in the precursor cells 6–8 h earlier. This time lag is caused by the maturation of precursors before they reach the circulation. Therefore, the 20 h reticulocyte RNA which was in the spleen 6–8 h earlier is still a product of continuous labelling.

Further data on the poly(A) size distribution was obtained by measuring the specific radioactivity of the short and longer poly(A) size classes (Table 1). The mole fraction of each size class is constant and reflects the steady state distribution of poly(A) sizes in reticulocytes. This is in agreement with the distribution of unlabelled mRNA on Millipore filters. The actual distribution of mRNAs containing each size class is identical at each time point. The only variation is the distribution of radioactivity and this represents the change of labelled poly(A) during the labelling period. This is further evidenced in that at 20 h the distribution of labelled poly(A) finally reaches that of total poly(A), that is, steady state. We therefore plotted the change in distribution of labelled poly(A) molecules (Fig. 2). The data are given as the fraction of labelled poly(A) in each size class with time. The 18-h time point is

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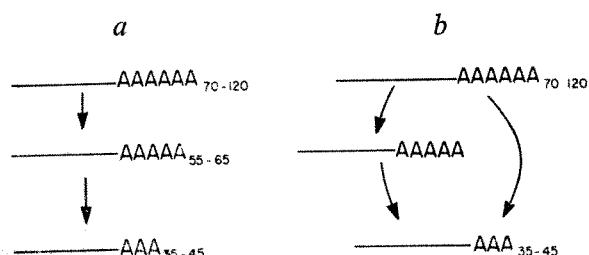


Fig. 3 Two models of poly(A) shortening. *a*, Sequential degradation model would be compatible with the data as a first order process if the curve for the smallest size class showed a sigmoid character before 6 h. *b*, Branched and sequential process would fit the data if the curves before 6 h were as presented.

from our previous data¹, and the 0-h values are based on the assumption that the change in size class distribution is the result of poly(A) shortening and not differential synthesis. This assumption has been validated by recent work using cultured mouse erythroid precursor cells which shows that the poly(A) sequence in newly synthesised globin mRNAs is a single size class approximately 150 nucleotides long⁸.

The decrease in the longer poly(A) size classes in globin mRNAs and the increase in the smallest size class can easily be seen. The data cannot distinguish, however, whether the largest size class gives rise to the intermediate size class alone or to both small and intermediate size classes. The curves before the 6-h time point can only be tentatively assigned because they will differ depending on which of the above two possibilities takes place. *In vivo* labelling experiments cannot provide data before 6 h as this time point is the earliest at which labelled globin mRNA can be isolated from reticulocytes. The observation, however, that at this earliest time point the majority of poly(A) fragments are in the smallest size class, indicates that the shortening takes place in the precursor cells as well as in reticulocytes.

We present two models for poly(A) shortening of globin mRNAs (Fig. 3). In the first model (Fig. 3*a*) each mRNA molecule is converted to the intermediate with the next longest poly(A) size class, whereas the second (Fig. 3*b*) allows for some mRNAs to have their poly(A) sequence shortened directly to the smallest size class. Further work on poly(A) shortening in erythroid precursor cells could distinguish between the two models.

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Normal synthesis, transport and decay of mRNA in the absence of its translation

MESSANGER RNA (mRNA) in the cytoplasm of eukaryotic cells constitutes a small fraction of the total RNA synthesised in the nucleus¹. This mRNA seems to be selected from the heterogeneous nuclear RNA (hnRNA) and transported to the cytoplasm where it decays much less rapidly than the hnRNA, which decays in the nucleus. In sea urchin embryos about 10% of the total RNA synthesised in the nucleus becomes cytoplasmic mRNA which decays 10 times less rapidly than the hnRNA. In L cells 2% of the RNA is transported and it decays 50 times less rapidly than hnRNA². Several proposed models implicate ribosomes or ribosomal subunits in mRNA transport or decay^{3–6}, and we have tested these possibilities in sea urchin embryos using pactamycin to inhibit the association of ribosomes with mRNA⁷ and precursor pool techniques worked out in this laboratory for measuring RNA metabolism². The results show that neither synthesis, transport nor decay of mRNA depend on or are regulated by concurrent protein synthesis.

Pactamycin (4 µg ml⁻¹) reduces protein synthesis in sea urchin embryos by more than 95% within 10 min. Polysomes dissociate during the same period. A typical effect of pactamycin is shown in Fig. 1 for polysomes extracted from embryos incubated for 2 h with 10 µCi ml⁻¹ ³H-adenosine. In the cytoplasmic extract of untreated embryos, most radioactive, high molecular weight RNA sediments with the polysomes on sucrose density gradients². In the pactamycin-treated embryos radioactive RNA sedimenting with the polysomes was reduced to the level found in a control extract treated with EDTA to dissociate polysomes. No significant radioactive RNA was associated with the monosomes in the pactamycin-treated embryos. These results establish that pactamycin inhibits protein synthesis and prevents the association of mRNA and ribosomes.

Pactamycin does not prevent the initial binding of mRNA to a ribosomal subunit in some cells⁸. We examined this possibility for sea urchin embryos, since the association of mRNA with even one ribosomal subunit might be sufficient to produce normal regulation of RNA metabolism. Cytoplasmic fractions were prepared from embryos incubated in ³H-adenosine for 30 min, when radioactive RNA is just beginning to appear in the cytoplasm, and for 240 min, when steady state incorporation of radioactivity into mRNA is achieved. These extracts were centrifuged on sucrose gradients for 15 h at 20,000 r.p.m. to sediment the monosomes and subunits to the centre of the gradient. In such conditions even a few per cent of the mRNA would be evident as a peak of radioactivity if it were associated

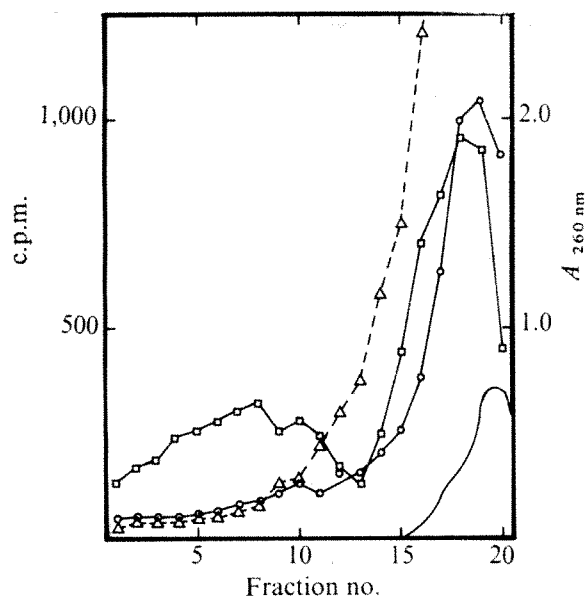


Fig. 1 Sucrose density gradient profiles of polysomes from control and pactamycin-treated sea urchin embryos. Three samples of 5×10^4 mesenchyme blastulae were labelled for 2 h with $10 \mu\text{Ci ml}^{-1}$ ^3H -adenosine. $4 \mu\text{g ml}^{-1}$ pactamycin was added to one sample concurrently with the isotope. The embryos were separated into nuclear and cytoplasmic fractions as before². Briefly, embryos were washed through dextrose several times to dissociate them into cells. They were lysed gently in detergent solution with a Pasteur pipette, layered over a sucrose cushion and centrifuged. This produces a pellet, which constitutes the nuclear fraction, and a supernatant above sucrose, which constitutes the cytoplasmic fraction. There is less than 1% of the total 28S and 18S RNA in the nuclear fraction, and less than 3% of the ^{14}C -thymidine-labelled DNA in the cytoplasmic fraction. All the radioactivity could be recovered in these two fractions. The cytoplasmic fractions were brought to 0.5% Nonidet P-40, one untreated sample was made 0.21 M in EDTA, and each was layered on a 36-ml preformed gradient of 15–35% sucrose in HSB buffer (0.43 M NaCl, 10 mM MgCl₂, 10 mM Tris-HCl, pH 7.4). Samples were centrifuged for 4 h at 24,000 r.p.m. at 4°C. Fractions were collected, and the acid-precipitable counts in each fraction were determined. \square , Untreated control; Δ , EDTA treated control; \circ , pactamycin-treated.

with ribosomal subunits (Fig. 2). There was no evidence of radioactive RNA associated with ribosomal subunits immediately after its synthesis or after longer periods in the cytoplasm. Hence, pactamycin prevents any significant attachment of ribosomal subunits to mRNA.

Pactamycin had no effect on the rates of synthesis and decay of total RNA. Sea urchin blastulae, 18–22 h after fertilisation, were incubated with $10 \mu\text{Ci ml}^{-1}$ ^3H -adenosine and samples were removed for RNA and precursor pool measurements. The amount of radioactive RNA that had accumulated after various times of incorporation was calculated from the radioactivity in the RNA and the specific activities of the ATP pools (Fig. 3). The radioactivity in the ATP pools differed slightly between pactamycin-treated and control embryos, apparently because DNA synthesis stops when protein synthesis is inhibited and this reduces the turnover of the ATP pool (Fig. 3, inset). The accumulation of radioactive RNA is not significantly different in normal and pactamycin-treated embryos; in both cases the RNA accumulates asymptotically to a steady state level in 5–6 h (ref. 2). The small difference between controls and treated embryos evident in Fig. 3 is the largest difference observed in any experiment. If synthesis is constant the decay curve is the inverse of the accumulation curve indicating that the decay of RNA must be essentially similar under both conditions. When the incorporation is initiated after a 4-h preincubation with pactamycin, the initial rate of accumulation of RNA is identical to controls, establishing that the instantaneous rate of RNA synthesis is not changed by pactamycin treatment. Therefore, the similarity in the accumulation

curves cannot be the result of pactamycin causing counterbalancing changes in the synthesis and decay of the RNA. About one-third of the total RNA accumulating in sea urchin embryos at this stage is hnRNA while the other two-thirds is mRNA. Since each of these two classes constitutes a significant portion of the total RNA, a change in synthesis or decay of either class would be readily apparent. It is clear that mRNA synthesis and decay remain normal when protein synthesis is inhibited by pactamycin.

Although the synthesis and decay kinetics of RNA did not change when initiation was inhibited by pactamycin, it was still possible that the transport of the newly synthesised mRNA to the cytoplasm was aberrant. In untreated embryos the percentage of radioactive RNA accumulating in the cytoplasm as mRNA increases asymptotically from a few per cent after a 15-min pulse to a steady state of 60% after several hours² (Fig. 4). Pactamycin had no effect on these kinetics of transport of newly synthesised mRNA to the cytoplasm (Fig. 4). As in normal embryos, the percentage of radioactive RNA in the

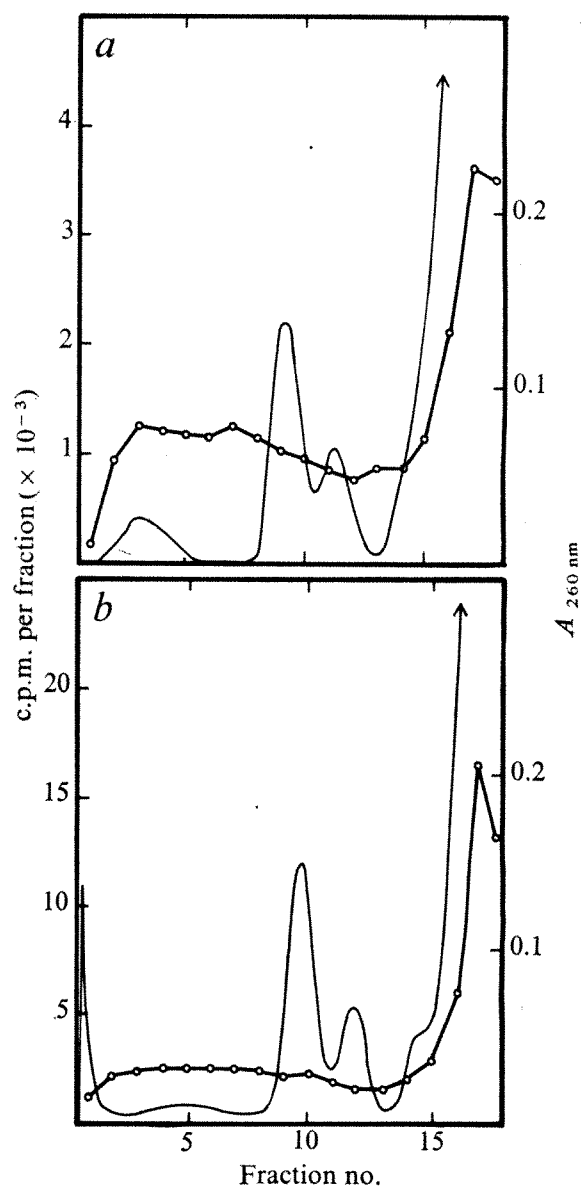


Fig. 2 Sucrose density gradients of the monosomes and ribosomal subunits. 5×10^4 Embryos were labelled and processed as described for Fig. 1. Cytoplasmic fractions were layered on sucrose density gradients and centrifuged for 15 h at 20,000 r.p.m. at 4°C, and fractions were processed as described. $4 \mu\text{g ml}^{-1}$ pactamycin and ^3H -adenosine were added concurrently for 30 min (a) and for 4 h (b). The solid line shows A_{260} .

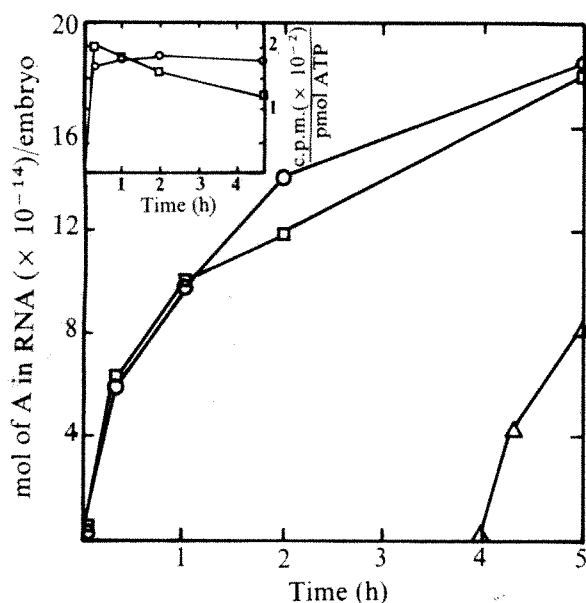


Fig. 3 Accumulation curves of radioactive RNA in the presence and absence of pactamycin. The methods for determining the accumulation curves for the synthesis of RNA have been described and justified previously². Briefly, embryos were labelled with ³H-adenosine, and a fraction was removed for RNA determination at each time point. Each fraction was homogenised in 0.5 N HClO₄ and the precipitate was collected by centrifugation and washed with 0.5 N HClO₄. The ATP was isolated from the acid extract of whole embryos, assayed by means of the luciferin-luciferase system, and counted for radioactivity. The acid-precipitable RNA was hydrolysed with NaOH and the alkaline-labile radioactivity in RNA and the alkaline-stable radioactivity in DNA were determined. The control sample (□) received no antibiotic; 4 μg ml⁻¹ pactamycin was added to one sample concurrently with the isotope (○) and to a second sample 4 h before addition of isotope (△). Inset: kinetics of incorporation of ³H-adenosine into ATP. The specific activity of the ATP pool was determined at each time point (see above). □, Control embryos; ○, pactamycin-treated embryos.

cytoplasm of pactamycin-treated embryos increased to about 60% in 4–5 h. This shows that mRNA is transported to the cytoplasm without associating with ribosomal subunits. Even though it accumulates there free of ribosomal particles and is not translated, it decays normally.

This normal synthesis, transport and decay of mRNA in the absence of protein synthesis indicates that translation is not

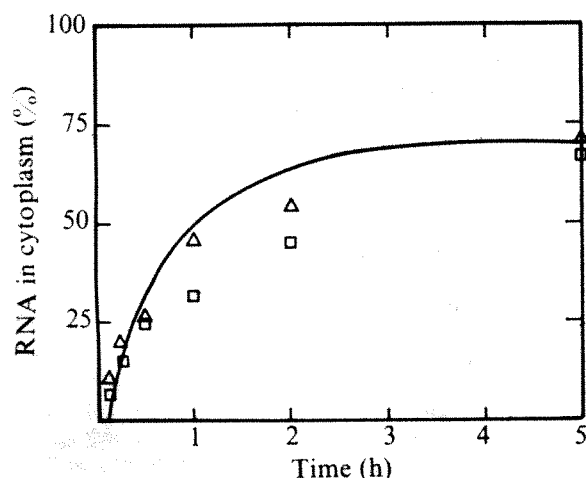


Fig. 4 Kinetics of transport of RNA to the cytoplasm. 10⁴ embryos were labelled and processed as described for Fig. 1 to yield nuclear and cytoplasmic fractions. Samples were hydrolysed in 0.3 N NaOH for 90 min, and the alkaline-labile radioactivity in RNA was determined. 4 μg ml⁻¹ pactamycin was added 2 h before label (△), or concurrently with label (□). The solid line shows the theoretical curve calculated from decay rates and steady-state levels of nuclear and cytoplasmic RNA².

involved in the mechanisms which control mRNA metabolism and that the proposed models that contain such mechanisms are not likely to be valid in sea urchin embryos. They also show that concomitant protein synthesis is not required for normal mRNA or hnRNA metabolism. If there are specific proteins normally associated with mRNA^{9,10}, these are either present in large excess, are recycled or have no role in regulating RNA metabolism.

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Binding of flexible ligands to macromolecules

MANY of the small molecules, such as enzyme substrates and inhibitors, hormones and neurotransmitters, the interactions of which with macromolecules are of fundamental importance in biology, may exist in solution in a number of conformations in equilibrium with one another. On the other hand, evidence from crystallographic studies suggests that when bound to a macromolecule these small flexible ligands have a single, well defined, conformation. The formation of such a ligand-macromolecule complex must therefore involve a process of conformational selection, which will influence the binding constant and the kinetics of complex formation. The apparent binding constant will simply be the product of the binding constant for the 'correct' conformation of the ligand and the mole fraction of this conformation in solution. The kinetic effects of conformational selection will depend on the mechanism of the binding process, and two contrasting models for this may be considered^{1,2}.

The first model is essentially an extension of Fischer's 'lock-and-key' model (Fig. 1a) in which only those ligand molecules which collide with the binding site in the correct conformation (and the correct orientation) are assumed to form a stable complex. In this single-step binding, all the favourable interactions between each 'segment' of the ligand and the corresponding 'subsites' on the macromolecules are formed simultaneously. The unfavourable losses of ligand entropy (translational, rotational and conformational) also occur in a single step. Thus the change in free energy on binding is given by

$$\Delta F = -RT \ln K = \sum_i \Delta H_i - T(\Delta S_{tr} + \Delta S_{rot} + \sum_i \Delta S_{conf,i})$$

(where ΔS_{tr} , ΔS_{rot} and ΔS_{conf} represent the changes in translational, rotational and conformational entropies of the ligand, ΔH_i represents the contribution to the enthalpic change from the binding of the i^{th} segment and the summation is over all 'segments' of the ligand). As for the kinetics of binding, in the absence of any orientational or conformational restrictions, we would expect the rate of binding to be controlled by diffusion. For the 'lock-and-key' model, however, we would expect the association rate to be less than this, as a given encounter will lead

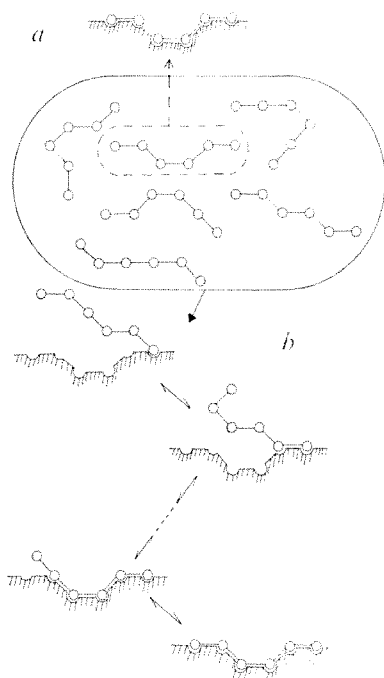


Fig. 1 Schematic representation of (a) 'lock-and-key' and (b) 'zipper' models for ligand binding discussed in the text. Only those ligand molecules which instantaneously have the correct conformation can bind as in (a), but essentially all ligand molecules can bind as in (b).

to stable binding only if the conformation of the ligand is suitable at that instant. In addition, this conformation must be presented in a suitable orientation, and this will further reduce the effective association rate constant. For a relatively large, flexible ligand, such as a peptide, this combination of conformational and orientational effects could easily lead to a reduction in the association rate constant by several orders of magnitude.

An alternative model is shown schematically in Fig. 1b. This model, which is similar to the 'zipper' model used in studies of double-helix formation in nucleic acids³⁻⁶, has been discussed previously⁷, and a detailed statistical-mechanical analysis of a distinct but related model has also been presented⁸. Here, we suppose that an initial, 'nucleation' complex can be formed by interaction of a single segment of the ligand with its subsite, and that this is followed by a series of conformational rearrangements of the partly bound ligand, leading to binding of the remaining segments to their appropriate subsites. The association rate constant for the formation of the initial complex will approach closer to the diffusion limit than that for the 'lock-and-key' model since the orientational and conformational requirements will be substantially less stringent. While the favourable enthalpy change on binding will be much less for this single-subsite interaction than for the binding of the whole ligand molecule, the loss of conformational entropy will also be considerably less (the changes in translational and rotational entropy being the same in both cases). Thus for the nucleation subsite

$$\Delta F_1 = -RT \ln K_1 = \Delta H_1 + T(\Delta S_{tr} - \Delta S_{rot} - \Delta S_{conf,1})$$

If this initial, single-subsite binding is to be followed by complete binding of the ligand to all the subsites, its lifetime must be long enough for the occurrence of the conformational rearrangements necessary for binding of an adjacent segment. Even if the lifetime of the complex is very short, say 10^{-7} s (corresponding to a binding constant $K \sim 10 \text{ M}^{-1}$ and an association rate constant $k_{on} \sim 10^8 \text{ M}^{-1} \text{ s}^{-1}$), this is still long compared with the time required for rotation about single bonds, which may occur at rates greater than 10^{10} s^{-1} . For the subsequent propagation steps, again only a partial loss of conformational entropy is

involved and here there is no loss of translational or rotational entropy. For the binding of the n^{th} segment

$$\Delta F_n = -RT \ln K_n = \Delta H_n - T\Delta S_{conf,n}$$

The fact that a smaller entropy loss is involved in propagation than in nucleation leads to a substantial 'cooperativity' in the binding⁷. In other words, there is a strong probability that nucleation will be followed by propagation, leading to complete binding.

Attempts have been made to define the conformational distribution of small molecules such as acetylcholine in solution^{9,10}, on the grounds that this is an important parameter in considering their binding to a receptor. As a corollary of this argument, it has often been assumed that the conformation of the ligand in the complex corresponds to the predominant conformation of the ligand in solution, although there is no *a priori* reason why this should be so, and indeed there are a number of instances where it is not true¹¹⁻¹⁴. If the conformation adopted by the ligand in the complex is one which is poorly populated in free solution, the 'lock-and-key' model predicts that the association rate constant will be low. On the other hand, for the 'zipper' model, since the rate of binding is likely to be determined by the nucleation step, the forward rate will be less dependent on the nature of the bound conformation. The 'zipper' model thus provides a mechanism for the rapid binding of ligands, even when a conformation of low population is involved.

The kinetic difference between these two models for ligand binding can be simply represented in terms of energy barriers (Fig. 2). In the 'lock-and-key' model there is a single large energy barrier (free energy of activation) for binding, containing translational, orientational and conformational terms. In the

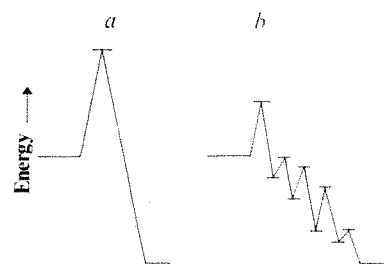


Fig. 2 Schematic representation of the 'reaction coordinate' for ligand binding by the 'lock-and-key' (a) and 'zipper' (b) models.

'zipper' model, this is replaced by a series of smaller energy barriers corresponding to the successive steps in binding, and since the overall rate is related to the height of the largest energy barrier in the reaction sequence, the association rate will be accelerated. As the two models only represent alternative pathways for going from the same initial to the same final state, the equilibrium constant must of course be the same for both models. Therefore, the faster association rate predicted by the 'zipper' model will be accompanied by a proportionately increased dissociation rate; again, only a series of relatively small energy barriers needs to be overcome.

The kinetic advantage of the 'zipper' model acquires additional interest when one considers the possibility of mutual conformational adjustment of both the ligand and macromolecule. In the 'zipper' model, this mutual adjustment can occur in a series of discrete, successive steps, between which the overall activation energy is partitioned. The overall process will thus, again, be significantly faster than in the 'lock-and-key' model. Since the activation energies for conformational changes in macromolecules may be considerable, a process which can lower these barriers may be expected to lead to a substantial acceleration of the overall rate of binding.

The two models presented here are obviously oversimplified and extreme cases. Behaviour intermediate between the 'lock-

and-key' and 'zipper' mechanisms is not only possible but likely. One might expect that molecules with limited degrees of conformational freedom would show behaviour closer to the 'lock-and-key' case, whereas very flexible ligands (such as linear oligopeptides) would bind in a 'zipper' fashion. There may also be cases where the energy barrier to a necessary conformational change is so high that the probability of its occurrence during the lifetime of the nucleation complex is very small¹⁵; the binding process in this case will tend to follow the 'lock-and-key' mechanism.

The development of the ideas presented here arose from our interest in the solution conformations of peptide hormones. These molecules seem to have a large number of conformations of similar energy, yet they are clearly capable of binding rapidly and specifically to a receptor. As pointed out by Eigen⁴, a mechanism for information transfer must satisfy the dual criteria of accuracy (specificity) and speed. High specificity is most rapidly achieved by having a relatively large recognition site (that is, a relatively large number of 'segments'). The 'zipper' mechanism affords a simple way to reconcile this requirement with that for rapid onset and offset of action.

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Cytoplasm-chromosome interactions in *Drosophila melanogaster*

INSTANCES of exceptionally high frequencies of mutation¹, male recombination², chromosomal aberration³ and sterility⁴ have been observed, often simultaneously⁵⁻⁸, in many natural populations of *Drosophila melanogaster* during the past thirty years. In some cases^{2,6,8}, these exceptional phenomena seem to be associated with chromosomes but not necessarily with specific

loci. Tests for an infectious agent have given negative results^{2,4}. Cytoplasmic factors, interacting with specific chromosomes, have been implicated in a few cases^{4,7} but in many others the experimental design did not provide for their detection. We report large differences in male recombination and F₁ sterility between reciprocal crosses of strains derived from temporally and geographically diverse natural populations and several commonly-used laboratory marker and balancer stocks. Cytoplasm-chromosome interactions are suggested.

The dictum that spontaneous crossing over does not occur in *D. melanogaster* males has been widely accepted since the early reports of Morgan⁹. Non-trivial frequencies are therefore indicative of unusual genetic events. We estimated the frequency of recombination in the third chromosome of F₁ males produced by mating males from each of twelve wild-type strains (Table 1) with females of the multiple-marked third chromosome stock *rucuca*¹⁰. The twelve strains are identified by the location and year of collection from the wild, if known. Since collection, the strains have been maintained by mass mating in half-pint milk bottles. Individual *rucuca*/+, F₁ males were mated with five virgin *rucuca* females in 8-dm vials. Progeny emerging from the first 9 d of egg production were scored. Temperature was controlled at 25 ± 1°C and a standard maize-meal-molasses-agar medium, seeded with live yeast, was used.

F₁ male fertility was normal in all *rucuca* crosses. The mean minimum percentage recombination is a conservative estimate of the frequency of independent male recombination events in the total length of the chromosome because all clusters of multiple recombinants, within the same interval of individual males, are counted as single events. Non-trivial frequencies of male recombination were observed in chromosomes which had been derived from natural populations within the last decade but not in those from longer-established laboratory stocks, differences between individual strains of the two groups are as great as two orders of magnitude. Differences among strains within the high recombination group are generally smaller but significant ($P < 0.001$).

Five of the strains which were tested for male recombination in the third chromosome were also tested with a second chromosome marker stock, MMIIa. This stock is homozygous for the markers: aristaless, *al* (2-0.01); clot, *cl* (2-16.5); black, *b* (2-48.5); curved, *c* (2-75.5); speck-2, *sp*² (2-107.0). F₁ males for recombination tests were produced in two ways: in cross A, wild-type males were mated with MMIIa females and in cross B, MMIIa males were mated with wild-type females. Individual MMIIa/+, F₁ males were, in each case, mated with five homozygous MMIIa virgin females and the frequencies of F₁ sterility and male recombination recorded (Table 2). In cross A all four strains which had exhibited male recombination in the third chromosome also did so in the second chromosome. Recombination in Oregon R-C was negligible in both chromosomes. In contrast, cross B male recombination was either absent or much reduced from the level observed in cross A. Moreover, F₁ fertility in both males and females was drastically reduced in cross A in the cases of Cranston, Harwich and Weymouth strains whereas, cross B fertility was normal in every

Table 1 Mean frequencies of sterility and third chromosome recombination in F₁ males from crosses between *rucuca* females and males from twelve wild-type strains

Strain	Male sterility	Total progeny	Minimum male recombination
	%		%
Oregon R-C, 1925	3.3	4,372	0.02
Princeton, 1929	0	4,965	0.02
Ames I, 1938	0	2,522	0.04
Canton S	4.0	2,568	0.04
Nettlebed	3.3	3,295	0.03
Ottawa, Ontario, 1962	3.3	4,982	0
Cranston, Rhode Island, 1964	2.6	9,593	1.39
Amherst, Massachusetts, 1966	5.0	4,534	0.26
Harwich, Massachusetts, 1967	3.3	4,664	2.19
Chepachet, Rhode Island, 1974	0	2,400	0.70
Weymouth, Rhode Island, 1974	5.0	3,129	0.35
Winnepesaukee, New Hampshire 1974	3.2	2,157	0.93

Table 2 Mean frequencies of F_1 sterility and second chromosome male recombination from reciprocal crosses between the MMIIa marker stock and five wild-type strains

Strain	Cross A*				Cross B†			
	F_1 female sterility %	F_1 male sterility %	Total progeny	Minimum (male) recombination %	F_1 female sterility %	F_1 male sterility %	Total progeny	Minimum (male) recombination %
Oregon R-C, 1925	6.7	0	1,825	0	0	0	1,710	0
Cranston, Rhode Island, 1964	46.0	57.8	3,020	0.83	0	0	4,449	0.07
Amherst, Massachusetts, 1966	ND	6.8	3,504	0.46	0	0	3,221	0
Harwich, Massachusetts, 1967	68.3	41.7	3,731	1.13	4.2	5.0	1,802	0.11
Weymouth, Rhode Island, 1974	20.0	9.1	3,451	0.32	0	2.0	3,024	0.07

* MMIIa female \times wild-type male.† Wild-type female \times MMIIa male.

ND, not determined.

case. Sterility is measured as the percentage of F_1 individuals which produce no offspring when mated with three or more members of the opposite sex. This is a conservative estimate because many of the individuals which were classified as 'fertile' in cross A produced considerably fewer progeny than their counterparts in cross B.

Male recombination and sterility continued through at least two succeeding generations of suitable back-crossing, at levels undiminished from those in the F_1 . In each of three generations, males heterozygous for Cranston and MMIIa, were back-crossed with MMIIa virgin females and their progeny tested as previously described. The results (Table 3) suggest that male recombination and sterility arise from interactions between chromosomes of some strains and the cytoplasm of others, each combination possibly exhibiting its own unique characteristics.

Reciprocal differences in both male recombination and sterility are not restricted to crosses with the MMIIa stock. Strains which produced high male recombination in the third chromosome (Table 1) showed greatly reduced frequencies in reciprocal crosses with the *rucuca* marker stock: the numbers of independent male recombination events were 0 in 1,848 progeny with Amherst, 1 in 5,678 (0.2%) with Cranston and 9 in 3,623 (0.25%) with Harwich.

Although *rucuca* crosses gave progeny with normal fertility irrespective of the direction of the cross, matings of the Cranston strain with several other, widely-used, balancer and marker stocks have yielded large reciprocal differences in sterility: for example, with the multiple balancer stock H-41 (ref. 11), F_1 sterility was 35.8% in cross A and 0.8% in cross B; with the *Basc* marker stock, over 50% sterility was observed in $F_1 \times F_1$ matings. Picard *et al.*¹² observed seemingly similar interactions between strains, resulting in female sterility.

Table 3 Mean frequencies of sterility and male recombination in three generations of backcrossing Harwich 1967/MMIIa males to MMIIa females

Generation	Female sterility %	Male sterility %	Total progeny	Minimum male recombination %
1	68.3	41.7	3,731	1.13
2	57.1	51.2	1,870	1.07
3	47.5	49.2	3,270	1.01

In summary, we have observed non-trivial frequencies of male recombination in F_1 males of crosses between second and third chromosome marker stocks and wild-type strains which had recently been collected from natural populations. Longer-established laboratory stocks did not exhibit this phenomenon to any significant extent. In susceptible strains, male recombination seems to be dependent on the direction of the initial parental cross. In at least three crosses, male recombination and sterility were highly correlated, both showing large reciprocal

differences in the same direction.

These results may have importance, not only for the understanding of the aetiology of these exceptional phenomena but may also have implications for reproductive isolation between sympatric populations. Moreover, if cytoplasm-chromosome interactions between laboratory marker and balancer stocks and other strains prove to be common, greater care will be necessary in the design and interpretation of experiments using such stocks.

This work was supported by grants from the National Institutes of Health and the National Science Foundation. We thank Ms Nancy Kessin for technical assistance, and Dr M. T. Clegg and Mr P. A. Fuerst for reading the manuscript.

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Errata

In the article "Evidence for a Y chromosomal contribution to an aggressive phenotype in inbred mice" by M. K. Selmanoff, J. E. Jumonville, S. C. Maxson and B. E. Ginsburg (*Nature*, **253**, 529; 1975) the column headings in Table 1 should read DBA/1 Fathers and C57BL/10 Fathers respectively, in both studies.

In the article "Earthquake simulation by nuclear explosions" by O. C. Kolar and N. L. Pruvost (*Nature*, **253**, 242; 1975) the following corrections should be made. Under the heading 'Seismic monitoring' the first sentence of para. 3 should read, "P-wave trains from an earthquake are often extended in time whereas those from an explosion tend to be more compact." Under the heading 'Seismic evasion', para. 11, line 1 should read "... Fourier spectra of the composite of ...". Under the heading 'Practical considerations', para. 2, line 4 should read "operation; including the conceptual design of a ..." and para. 10, line 5 should read "... depth is in the range of 120W^{1/3} m (ref. 5)."

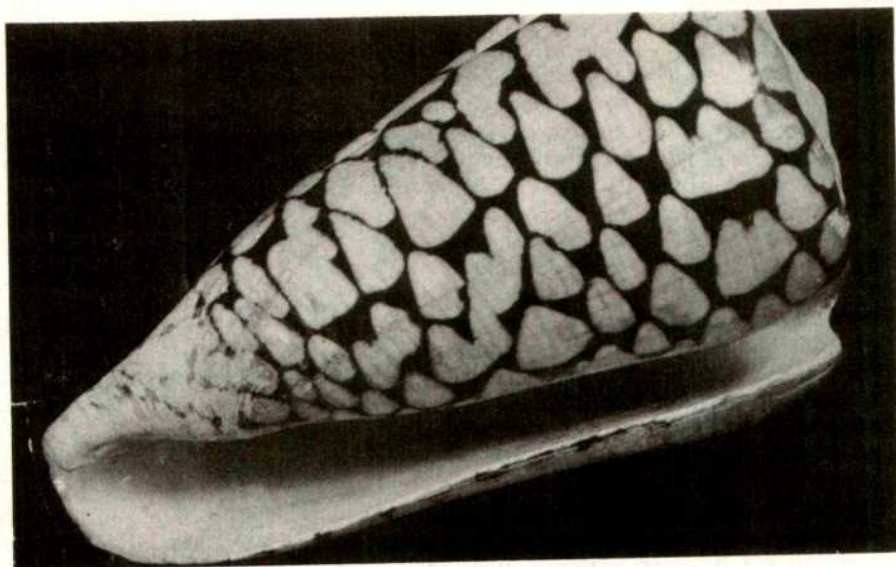
reviews

THE Mollusca are surely the most interesting of the invertebrate phyla. They are unique in producing a shell which may be considered independently of the animal which formed it, so that their study has been divided between conchologists and malacologists. True, the acumen of Aristotle enabled him to view the molluscs as a whole, apart from a not unnatural assignment of the cephalopods to a higher rung in his ladder of life, but this unity disappears among his successors.

Enlargement of the world at the renaissance led to the accumulation of shells from all oceans and it was these, beautiful in themselves, indestructible and eminently suitable for cabinet display, which occupied the attention of Martin Lister in the 17th century. Such names as he and others bestowed became incorporated into the binomial nomenclature of the *Systema Naturae*. But Linnaeus was content to group the animals into ten 'genera' and his followers, after describing the shell, merely referred "its animal" to one of these. All shelled species became included in the Testacea; only the shell-less slugs were classed as Mollusca which embraced a vast disorder of worms, zoophytes and echinoderms.

Malacology as such emerged from the comparative anatomical studies of Cuvier, which revealed the basic molluscan plan that is often ingeniously disguised because of the unique plasticity of the phylum. During the last century, as knowledge about the molluscan animal increased it was alongside a world wide search for shells described in numerous, often magnificently illustrated, shell iconographies.

This century has seen impressive progress in molluscan studies covering every aspect from population dynamics to the finest structural details revealed by the electron microscope. Of recent years there has been a revival of popular interest in shells, which has led to, and been further stimulated by, the publication of a series of impressive volumes, often with magnificent coloured plates, coming largely from the United States and Japan. Only rarely, however, as in Wilson and Gillett's *Australian Shells*, do animals themselves receive adequate attention. And



Suitable for cabinet display

it is with the laudable hope of remedying this lack of general knowledge about the living animal that Dr Alan Solem, one of the most rigorous contemporary malacologists whose activities are centred on, but far from confined to, the Field Museum of Natural History at Chicago, has written this appropriately named book*.

He should certainly succeed in his object. This is a very attractively produced and most excellently illustrated book which cannot but increase the knowledge and molluscan interests of readers ranging from the amateur conchologist to the professional zoologist. Naturally, there are inequalities in knowledge and presentation. The extraordinary plasticity of molluscs has been responsible for their unrivalled diversity of form and of habit. No one is likely to be equally conversant with limpets and squids and be as knowledgeable about the environment of abyssal seas as about that of deserts. Moreover, those who study marine molluscs seldom have time to concern themselves with terrestrial species, and the reverse is no less true.

Dr Solem is a member of the latter fraternity and in his chapters dealing with land molluscs, a preoccupying abundance of some 24,000 species, he writes with impressive authority. We encounter the fascinating devices for reproduction such as those in *Limax cinerioniger* which is five to eight inches long but in which the genitalia, protruded by the intertwining pair, reach

lengths of up to 32.5 inches; he also covers adaptations for survival in desert climates or over long dry periods, and the course of evolution, as usual in molluscs from many converging origins, of the land slugs which have largely ceased to be 'shell makers'. There is an altogether admirable chapter dealing with how "To scrape a Living", concerned with the radula and illustrated with 21 plates including scanning electron microscope photographs that reveal the structural diversity of this wonderfully adaptable tool which has been so very significant in the success of the phylum.

Dr Solem has been too deeply preoccupied with land mollusca and snails to have the same commanding knowledge of marine species. He does not attempt to describe the ctenidial gills, organs as supremely successful as the radulae which, indeed, they supercede as organs of feeding throughout the Bivalvia in which group, it must be noted, the palps do not (as is claimed in the book) push food into the mouth. Nor can I follow Dr Solem in his treatment of the vexed problem of torsion.

But this is a revealing book for all readers, enriched by appendices covering classification, references for further reading, a glossary, and some excellent advice about the care and feeding of both marine and freshwater molluscs, and of land snails and slugs. In all, a most welcome addition to molluscan literature.

C. M. Yonge

**The Shell Makers*. By G. A. Solem. Pp. 280. (Wiley: London and New York, June 1974.) £5.05.

Instant zoogeography

Aspects of Zoogeography. By Paul Müller. Pp. viii+208. (Junk: The Hague, 1974.) Dfl. 35.

PAUL MÜLLER is known for some interesting work on Brazilian reptiles and the zoogeography of South America. His latest work, entitled *Aspects of Zoogeography*, runs briefly through definitions of zoogeography and the biosphere. It summarises the characteristics of zoological realms, there are brief sections on each of nine terrestrial and two freshwater biomes. It contains a trendy little chapter on urban ecosystems. And the book ends briefly with historical aspects of zoogeography. All this in 170 pages, half of them occupied by illustrations.

It is difficult to see how some of the diagrams are related to the text and many of the diagrams are difficult to understand because they are too complex or the captions fail to describe them.

In the brief text, there is little discussion. For example, continental drift is mentioned but its relevance to animal distribution is not considered. The desert lizard *Uromastix* is listed as having salt glands but the significance of salt excretion for desert lizards is not discussed.

The book could be useful to a student

who wants a synopsis of zoogeography. The references are there and the bibliography lists German works in addition to more familiar English and American works.

But the book is unsatisfactory because there is no assessment of the facts; and no personal opinion emerges, except for the assertion that the dispersal centre concept of Reinig and de Lattin should be given more attention. But it would be necessary to turn to de Lattin's work to discover the implications of that.

The book has been incompetently edited. There is no excuse for so many misprints.

Wilma George

Colour and function of pigments

The Significance of Zoochromes. By A. E. Needham. Pp. xx+429. (Springer: Berlin and New York, 1974.) \$26.50.

THE pioneer investigations of natural pigments by C. A. MacMunn at the end of the last century did not stimulate much interest among his contemporaries, and it is only within the last 25 years or so that his work has received the attention it deserves. Even now, however, researchers who have their main interest in pigments in their own right form a small and exclusive band.

Dr Needham's book is doubly welcome, for it not only considers animal pigments from the points of view of structure and function, but it also presents information and concepts which have never been assembled in one volume before. A discussion of the molecular basis of colour and the chemistry of the natural pigments is followed by the main part of the book which deals with the functions of these pigments. Biochemical and physiological rôles are considered, leading to an assessment—supported by evidence from metabolic pathways, from evolution and from the genetic basis of pigment formation—of the significance of the pigments in animals.

The format of the book is very pleasing, with particularly high quality typeface and paper. It was a good idea to preface each chapter with a brief synopsis of its subject matter and to include a conclusion section at the end. There are many tables which collect data not easily found elsewhere: the discussion of the taxonomic distribution of pigments with its accompanying table is a good example. The chapters dealing with integumental pigments and camouflage and the biogenetic evidence for their significance are of great interest, and the final chapter, a general assessment, contains

much that is controversial. The list of references is comprehensive and most useful.

Unfortunately, the book contains many errors and misguided attempts to change well-tryed and familiar nomenclature, which will make it irritating to those who have spent many years in this field. The title itself is provoking—why not 'Animal Pigments'?—'pigment' is a much better word than the rather pompous 'chrome', which, in any case, suggests the metal. To use the word 'porphyran' for 'metalloporphyrin' is misleading (*malgré* Fearon), and suggests a false relationship with porphyrin. The words 'glycose' and 'alkanol' are based on false logic. Use of the word 'chromasome' to describe a pigment granule leads to a hopeless confusion, both written and spoken. There is a daunting list of quite superfluous abbreviations at the beginning of the book.

Straightforward errors are more serious, and space allows mention only of the very worst. The use of the word 'chloroporphyrin' when 'chlorocruoroporphyrin' is meant is an illustration of the way in which obstinate pedantry reaps its own reward. Svedberg divided invertebrate haemoglobins into erythrocruorins and chlorocruorins for excellent and sound reasons, and it is chlorocruorin which gives rise to chlorocruorohaem and chlorocruoroporphyrin. Chloroporphyrin is a tricarboxylic derivative of chlorophyll. Phthalocyanins do not contain indole nuclei but are tetraporphyrins in which the methene bridges are replaced by tertiary N and the four pyrrol rings are condensed with benzene rings in their β -positions. Fossil porphyrins do not always lose their COOH groups. Solubility data, fluorescence, and the formulae for many porphyrins are often wrong. The important feature of the Soret band in the porphyrin absorption spectrum is that it is dependent upon the integrity of the macrocyclic ring (not "super-ring").

There are many omissions of important material which one would expect to find in a book of this kind. They include Warburg's ideas about primitiveness and the formyl group; mention of the pigments of birds' eggshells; the photodynamic work of Meyer-Betz, Boyd and Gaffron with porphyrins and proteins; and discussion of the effects of substituent groups and metals on the porphyrin absorption spectrum.

The author has striven too hard to achieve absolute exactness, and this has led him into pedantry and often downright error. Even as it stands, however, the book is a valuable and unique contribution to the literature on pigments.

G. Y. Kennedy

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Sweet review

Sugars in Nutrition. (The Nutrition Foundation: A Monograph Series.) Edited by H. L. Sipple and Kristen W. McNutt. Pp. xx+768. (Academic: New York and London, November 1974.) \$45.00; £21.60.

THIS powerful and informative book is based on a number of papers presented at the International Conference on sugars held at the Vanderbilt University School of Medicine in 1972, at which world authorities within the field were very well represented.

The book commences with an historical account and general synopsis of sugars and nutrition, along with psychological and biochemical descriptions of their sweetness. A useful account of their occurrence and incorporation in foods is followed by some statistics of their use and recent technological advances. An entire section on the metabolism of sugars includes also some effects attributable to polyols, especially sorbitol and xylitol which are now so important as food additives and which are still, of course, items of modern therapy. This section includes a valuable paper from Macdonald on the metabolic effects of maltose and higher oligosaccharides, which are now being increasingly used by food manufacturers as a substitute for sucrose. A large section on disorders of sugar metabolism includes papers on inborn errors, sugar cataract, intolerance, hypertriglyceridaemia, cardiovascular disease and diabetes, and a section on therapy naturally includes some special emphasis on the uses and dangers of xylitol and mannitol in different forms of treatment. Finally, a section on the oral cavity deals with sugars, dental plaque and oral streptococci.

It is inevitable that a book of this magnitude should possess one or two puzzling features. Some contributors, for example, could have been expected to cover areas which have in fact been covered by different authors. Shallenberger, who has made one of the most radical contributions to the theory of sweetness in the last decade does not write either of the two papers on that subject. Nor is the major part of his work alluded to by the other two authors. It is, however, evident that some speakers at this symposium presented more than one work and that not all of these were selected by the editors.

Some of the newest and less well known research into sugars and nutrition does not seem to have been included in the book. Examples include the research by the National Aeronautics and Space Administration into the conversion of human waste products to formose sugars and their metabolic fate after re-feeding, and the

work at MIT on carbohydrates and brain metabolism. The effects of sugars on human work performance and fuel reserves seem also to have been excluded from discussion.

Any multidisciplinary scientific volume is liable to have its share of editorial mistakes and omissions, but of particular gravity are those on pages 153 and 155; the pyranose sugar structures depicted there are not the sugars they are claimed to be. Furthermore, the diagram of transport across a biological membrane gives the completely erroneous impression that D-glucose enantiomerises in the process. In spite of these faults, however, the book remains the best work to date for co-ordination and summary of the status of sugars in modern nutrition and physiology.

G. G. Birch

Sizing it up

Particle Size Analysis. (Series in Analytical Chemistry) By Z. K. Jelinek. Pp. 178. (Ellis Horwood: Chichester, 1974.) £5.00.

THE quantitative determination of the size, shape and distribution of particles in disperse systems is of considerable importance to the study of fogs and smokes, foams and emulsions, suspensions, porous solids and catalysts, and in general to systems consisting of a disperse and a continuous phase.

Particle sizes may vary from those found in polymer and colloidal solutions to those found in coarse dispersions, and as a consequence, a range of methods is necessary for the analysis of the different systems. This book gives a comprehensive account of the practical aspects of size analysis in these systems, and describes in detail the methods of measurement of size, shapes and pores in disperse systems, the measurement of surface areas by adsorption methods, and molecular weight measurements in polymer and colloidal solutions.

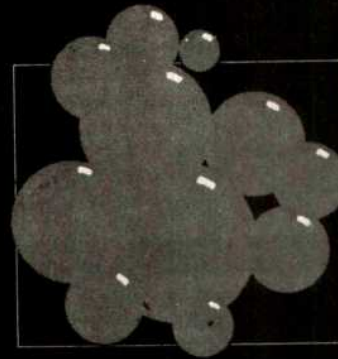
The coverage of the various methods is thorough, and the volume includes separate chapters on optical procedures (electron microscopy and ultramicroscopy, light scattering and X-ray diffraction), mechanical methods, gravitational and osmotic methods, viscosity and permeation, pore size analysis, and adsorption and conductometric measurements. The apparatus, technique and procedure is given in detail for each method, followed by a valuable selection of worked out examples. The references cover literature up to 1972.

This is a valuable reference work for chemists who desire a survey of the practical laboratory method of particle size analysis of dispersions.

C. E. H. Bawn

Chemistry books

1975



Molecular Behaviour and the Development of Polymeric Materials

Edited by A. LEDWITH and A. M. NORTH
January 1975: 562 pages: illustrated: 412 12400 9:
hardback: £12.00

This book opens with articles on the nature of ionic polymerizations and on the recently characterized role of intermolecular complexes in determining the course of polymerization reactions. Succeeding chapters consider topical aspects of general and specific applications of the main classes of polymers, and the book concludes with five reviews which discuss macroscopic physical and mechanical properties in the light of recent studies in molecular behaviour.

Growth of Crystals from the Vapour

M. M. FAKTOR and I. GARRETT
December 1974: 310 pages: illustrated: 412 11320 1:
hardback: £7.00

The text of this book starts with an introduction to crystal growth, followed by preparatory chapters on the thermodynamic basis of chemical vapour transport, and a discussion of crystallization and nucleation. Transport in the gas phase is then discussed from a simple theoretical standpoint. Interplay of transport and surface kinetic limitations and their influence on interfacial stability and electronic properties are explored next. The final chapters include a review of recent experimental exploration of some interesting concepts, and suggestions of where further effort could be applied.

Chromatographic Methods

Third edition
R. STOCK and C. B. F. RICE
Hardback: Third edition December 1974: 400 pages:
illustrated: 412 10560 8: £5.25
Science Paperback: 412 20810 5: £2.90

This new edition has been thoroughly updated incorporating the many new advances in the subject which have been developed since 1967. An attempt has been made to give some guidance as to the choice of method for a particular purpose and to assess the relative values of the different procedures.

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"Multiplication from a statistical viewpoint

Mathematical Models of Conception and Birth. By Mindel C. Sheps and Jane A. Menken. Pp. xxiii+428. (University of Chicago: Chicago and London, 1973.) £9.25.

In the last two decades a new approach has thrown light on the biological factors underlying the statistics of human reproduction. It is an approach which involves considering a sequence of stages in the reproductive process (such as a period of susceptibility to conception, pregnancy, or post-partum sterility).

Mathematical models play an important role in these studies mainly because such models offer a way of dealing with quantities which are not directly measurable; for instance, the probability of conception in one menstrual cycle, or, to take an even more intractable example, the distribution of such probabilities over populations of women. By using mathematical models and computer simulation, it is possible to relate assumptions about quantities not accessible to direct measurement to observable quantities

such as birth rates, intervals between births and so on. The study of such models produces a number of surprising results. For example, contraception which reduces the probability of conception among women at risk by $x\%$, will not reduce the number of births by $x\%$, but by much less. Thus, an average contraceptive effectiveness of well over 60% will probably be required to reduce births by 40%.

The work under review is a careful, detailed, clear and authoritative survey of this fast expanding subject. It is fully up to the high standards set over three decades by the publications of the Princeton Office of Population Research. The work is published as a posthumous memorial to the senior author, Mindel Sheps, who made fundamental contributions in this field of research. She died just before publication of the book.

The book offers more than the title promises. The mathematical analyses of a great variety of models are discussed. Extensive tables are given, showing the results of varying the parameters which enter into the models. This kind of material was to be expected. In addition, however, the book contains expositions of the parts of probability theory on which the models are based. The reader unacquainted with renewal theory, Markov chains or Markov renewal processes will find an introduction here. There is also coverage of basic distribution theory and various mathematical techniques so that in principle a reader equipped only with calculus and a basic course in mathematical statistics could use this book. In effect, it may be viewed as a text of much of applied probability theory (and some statistical theory) with detailed applications in one special area. As befits a mathematical textbook, there are worked examples and exercises for the reader at the ends of chapters, with answers at the back of the book.

What the reader will not find (with occasional exceptions) are examples of real data to the analysis of which these models can be applied. For example, a long chapter is devoted to models of the intervals which elapse between marriage (or some other suitable initial point of observation) and the first conception or first birth. The authors justly remark, at the beginning of this chapter, that "data on conception times and related phenomena have been used a great deal both in investigations of the biological basis of reproduction and in efforts to evaluate the effectiveness of contraception". Yet not a single recorded distribution of intervals to the first conception or birth is actually shown, nor are references given to publications where such data may be found.

Paradoxically, this lack of contact with reality may increase the sale of the book. For it can probably be used in courses on applied probability theory and stochastic processes given by lecturers with no experience in analysing demographic data. Common interest in, and common-sense knowledge of, sex and reproduction are all that is required.

The book may strengthen the suspicion (which is unjustified in this case) that probabilistic models are the toys of mathematicians and computer specialists not seriously concerned with the phenomena on which the models are expected to throw light. **J. Hajnal**

Molecular collisions

Molecular Collision Theory. (Theoretical Chemistry: A Series of Monographs.) By M. S. Child. Pp. 300. (Academic: London and New York, September 1974.) £8.50; \$22.00.

In recent years rapid progress has been made in the experimental investigation of molecular scattering. These experiments are a very important source of information about the potential surfaces on which it is supposed that nuclei move. Molecular collisions may conveniently be classified into three broad classes—elastic, inelastic and reactive. Each class is sensitive to a particular aspect of the potential surface. This book is intended as an introduction to the theory required for the interpretation of elastic, inelastic and reactive scattering experiments in chemistry. Emphasis is placed on the analytical treatment of quantum mechanical and semiclassical aspects of molecular scattering.

Chapters 2–5 present the quantum-mechanical and semiclassical theories of elastic scattering. They include such important topics as the Born approximation, the semiclassical phase shift, rainbow and glory scattering and orbiting. The theory of inelastic scattering is expounded with the aid of the scattering matrices in the next four chapters. The final chapter is devoted to reactive scattering. There are five appendices on relevant mathematical topics, including continuum wavefunctions and classical mechanics.

In the selection of material emphasis has been placed on obtaining analytical expressions of practical value to those involved in interpreting chemical experiments. The book is very successful in this respect and is likely to be popular with research students and others. It is authoritative, penetrating and compact. Although there are a number of minor errors I strongly recommend the book. **A. D. Buckingham**

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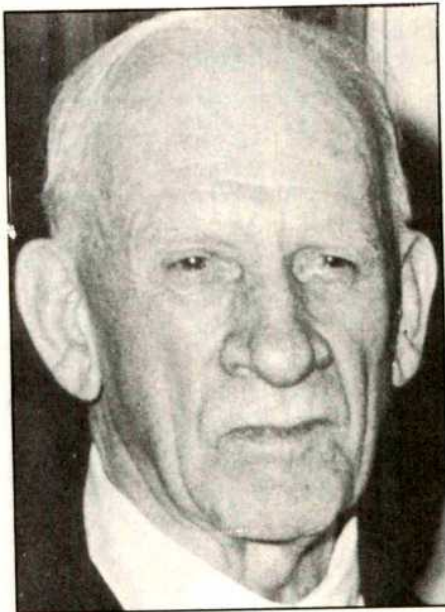
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obituary

Robert Robinson was born in 1886 at Chesterfield in Derbyshire and went to the University of Manchester to study chemistry, where he came under the influence of W. H. Perkin, Jr, a brilliant organic chemist then at the height of his powers and in charge of a famous research school: his Manchester contemporaries included W. N. Haworth, J. F. Thorpe, J. L. Simonsen and Chaim Weizmann who became his lifelong friends. Robinson became a colleague and close friend of Perkin and they collaborated in a series of beautiful researches on the dyewood colouring matters, brazilin and haematoxylin, and on alkaloids. When only twenty-six years old he was appointed to the chair of organic chemistry in the University of Sydney, a position he held for three years until appointed professor at Liverpool in 1915. During his short stay in Sydney, and despite the upheaval of moving across the globe and the complication of a disastrous laboratory fire which destroyed many of his records, Robinson pushed ahead with his work on alkaloids and especially with his ideas on biogenesis based on structural relationships in the alkaloid group. His theories were given a tremendous boost by his synthesis of the alkaloid tropinone by the interaction, in dilute aqueous solution at room temperature, of three simple molecules all of which could well be plant metabolites. His paper on tropinone published in 1917 not only led to widespread adoption of his biogenetic theories as a tool in natural product studies, but provided the primary stimulus to all the modern developments in the elucidation of biosynthetic mechanisms.

Colour in nature had fascinated him from the time of his early work on the dyewoods, and, perhaps spurred on by the fact that he was a keen gardener, he turned his attention to the anthocyanin pigments of flowers. Already a master of the classical degradation methods of the organic chemist, he introduced a new approach in his anthocyanin studies—the use of analogue synthesis as a powerful tool in structure determination—which influenced the pattern of much future work in the natural product field. The total synthesis of all the major anthocyanins allowed him to initiate work on the genetics of flower colour variation; it also provided chemists with some new synthetic methods which, together with many others he devised and exploited during



Sir Robert Robinson

An appraisal by Lord Todd

his career, have greatly enriched the synthetic chemist's armoury of weapons.

His natural product work and especially his biogenetic theory have had a great and lasting impact. Equally vital to the development of organic chemistry, is his work on the electrochemical theory of organic reactions. Robinson's ideas on the nature of reactivity in organic compounds were stimulated and reinforced by his association with Lapworth in Manchester during the 1920s, and it would be fair to say that what is often called the Lapworth–Robinson theory of organic reactions expressed by them in terms of the electronic theory of valence, and powerfully developed by Robinson, provided an essential stimulus to all the great advances made since that time in our understanding of reaction mechanisms in organic chemistry. The electronic theory developed, however, when ideas of atomic structure were still rather primitive, but Robinson's views, re-interpreted in terms of modern molecular orbital theory remain valid to this day.

All these contributions were made during a career in which he occupied in quick succession five chairs of organic chemistry (Sydney, Liverpool, St Andrews, Manchester and University College, London) and one research directorship in industry (British Dyestuffs Corporation 1920–21) before he succeeded his old teacher Perkin as Waynflete Professor at Oxford in 1930,

a position he occupied until his retirement in 1957. Although his stay in industry was short, he made a profound impact not just on the British Dyestuffs Corporation but throughout his career, as consultant and adviser, on the chemical and pharmaceutical industries; he joined the Shell Chemical Company after his retirement from Oxford and continued to be associated until his death. Even though failing physically, he maintained the amazing clarity of mind for which he was famous.

As would be expected, honours were heaped on him by universities and learned academies all over the world. He served as President of the Chemical Society (1939–41), of the British Association for the Advancement of Science (1955) and of the Royal Society (1945–50). He was awarded the Nobel Prize for Chemistry in 1947 and for his services to science in Britain he was knighted in 1939, receiving the Order of Merit in 1949. He was twice married—his son and daughter, and his second wife, survive him.

His career would seem to leave little time for other activities. But Robert Robinson was no ordinary man. Although deflected from it at university he maintained a lifelong interest in mathematics. Perhaps related to this was his passion for chess of which he was a first-class exponent, having the entrée to the major chess-clubs in any city which he visited both here and abroad. He was also fond of music and was a much better pianist than he would admit. Out of doors he found recreation in mountain climbing and walking and spent his summer vacations traversing the Alps of which he had an encyclopaedic knowledge. A man of great physical and mental toughness he was nevertheless a shy man whose manner with strangers could sometimes be for this reason brusque or withdrawn. To those who knew him he revealed himself as he really was—kindly, generous and loyal. Humble in the face of nature, he loved children and perhaps for that reason was loved by them. He enjoyed the cut and thrust of scientific argument but he could on occasion be a little brusque with, or— which was more devastating—simply ignore those who were, in his view, being obtuse. Few men have inspired greater respect and real affection among those who were privileged to work under his guidance and to gain his friendship. With his passing Britain and indeed the world has lost one of the greatest men of science of this century.

announcements

Awards

Hollis D. Hedberg has been awarded the **Wollaston Medal** by the Geological Society for contributions to micropalaeontology, stratigraphy and petroleum geology.

John Sutton has been awarded the **Murchison Medal** by the Geological Society for contributions to pre-cambrian geology, particularly in the Scottish Highlands and Islands.

Dorothy Raynor has been awarded the **Lyell Medal** by the Geological Society for contributions to vertebrate palaeontology and carboniferous geology.

Drummond H. Matthews has been awarded the **Bigsby Medal** by the Geological Society for his part in the Vine-Matthews hypothesis.

For his contribution to elementary particle physics, **Nicholas Kemmer** has been awarded the **J. Robert Oppenheimer Memorial Prize** by the Center for Theoretical Studies, Miami, Florida.

Appointments

Clifford Butler has been appointed Vice-Chancellor of Loughborough University of Technology.

Norman Blackburn has been appointed to the chair of pure mathematics in the University of Manchester.

Miscellaneous

Roussel Prize. Applications are invited for the Roussel Prize, awarded every two years to stimulate pharmaceutical research. The next prize (\$10,000) is scheduled for June, 1976 and will be concerned with work published before December, 1975 on steroids and related compounds. Nominations with the names of two referees, must be submitted before March 1, 1976. Further information from: The Secretary, Centre de Recherches, Roussel Uclaf, 93230 Romainville, France.

Theoretical Chemistry. The Charles Coulson Summer School in Theoretical Chemistry will be held at Oxford University from September 7-20, 1975. For further details, contact: Dr M. S. Child, Theoretical Chemistry Department, 1 South Parks Road, Oxford OX1 3TG, UK. Closing date: May 15, 1975.

Person to Person

Philosophy of Medicine. Contact wanted with people working in philosophy of medical science, especially the concept of disease, cause and the relation between the foundations of medical science and the health system. We are teaching a course 'The Theory and Philosophy of Medicine' at the University of Copenhagen. For information contact: Anders Ottar Jensen, Department of Biophysics, Juliane Maries Vej 28, DK 2100 Ø, or Hans Siggaard Jensen, Department of Philosophy, Købmagergade 50, DK 1150 K, Denmark.

Reports and publications

Great Britain

Energy Conservation in the United Kingdom: Achievements, Aims and Options. (A report by the National Economic Development Office.) Pp. viii + 106. (London: HMSO, 1974.) £3.40. [21]

Bulletin of the British Museum (Natural History). Zoology, Vol. 27, No. 7: A New Family, Genus and Species of Bat (Mammalia: Chiroptera) from Thailand. By J. E. Hill. Pp. 301-336. (London: British Museum (Natural History), 1974.) £2.10. [31]

Centre for Overseas Pest Research. Report, January-December, 1973. Pp. vi + 141. (London: Centre for Overseas Pest Research, College House, Wrights Lane, W8, 1974.) £1.30. [61]

Science Research Council. Telecommunications. Pp. 24. (London: Systems Engineering Committee Secretariat, Science Research Council, State House, High Holborn, W.C.1, 1974.) gratis [81]

A Park System for Scotland. Pp. 36. (Battleby, Redgorton, Perth: Countryside Commission for Scotland, 1974.) [91]

Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 269, No. 900: A New Genus and Species of Anthracoxaur Amphibian from the Lower Carboniferous of Scotland and the Status of *Pholidogaster pasciformis* Huxley. By A. L. Panchen. Pp. 581-640. (London: The Royal Society, 1975.) UK £2.40; Overseas £2.60. [91]

Broadcasting: The Developing Technology. By James Redmond. (BBC Lunch-Time Lectures, Ninth Series, 2.) Pp. 20. (London: BBC, 1974.) [141]

New Science in the Solar System. (A New Scientist Special Review. (Pp. 64 (55 plates.) (London: New Science Publications, 1975.) £1. [141]

National Radiological Protection Board. NRPB-R27: Factors for Deriving Absorbed Dose rates in Air due to Beta Particles from Measurements of Absorbed Dose-rates in Tissue-equivalent Material. By T. M. Francis and E. A. Pook. Pp. 12. NRPB-R28: The Use of Plutonium-239 Sources in Schools and Other Educational Establishments. By T. G. Williams. Pp. 18. (Harwell, Didcot, Oxon: National Radiological Protection Board, 1974.) [151]

Cancer Biochemistry Biophysics, Vol. 1, No. 1, 1974. Pp. 1-56. Edited by Harry Darrow Brown, in association with Robert C. Hahn. Subscription Rates (per volume, post paid). Six issues per volume, published bi-monthly. Great Britain: Personal subscribers who warrant that the journal is for their own use, and order direct from the publisher £6. Librarian, Institutions and other subscribers £22.75. (London and New York: Gordon and Breach Science Publishers, Ltd., 1974.) [161]

Whale Campaign Manual 2. Pp. 132. (London: Friends of the Earth, 9 Poland Street, W1, 1975.) 80p plus 20p postage. [161]

The Commonwealth Forestry Institute, University of Oxford. Fiftieth Annual Report, 1973/1974. Pp. 40. (Oxford: The Commonwealth Forestry Institute, The University, 1974.) [201]

Freshwater Biological Association. Scientific Publication No. 29: Turbulence in Lakes and Rivers. By I. R. Smith. Pp. 79. (Ambleside, Westmorland: Freshwater Biological Association, 1975.) £1. [201]

Other countries

World Health Organization. Manual on Radiation Protection in Hospitals and General Practice. Vol. 1. Basic Protection Requirements. By C. B. Braestrup and K. J. Vikterlof. Pp. 81. (Geneva: WHO; London: HMSO, 1974.) Sw. fr. 12. [1812]

Societ Biomedical Institutions: a Directory. (A Publication of the Geographic Health Studies Program of the John E. Fogarty International Centre for Advanced Study in the Health Science.) DHEW Publication No. (NIH) 74-698. (Washington, DC: US Government Printing Office, 1974.) \$5.95. [1812]

Annals of the South African Museum. Vol. 66, Part 4: Type Specimens of Decapoda (Crustacea) in the Collections of the South African Museum. By Brian Kensley. Pp. 55-80. R.4. Vol. 66, Part 5: The Cretaceous Stratigraphy of South-Central Africa. By Michael R. Cooper. Pp. 81-107. R.4.30. Vol. 66, Part 6: The Zoogeography of the South African Avifauna. By J. M. Winterbottom. Pp. 109-149. R.7.50. Vol. 66, Part 7: Pelt Skin Dressing Technique. By E. M. Shaw and H. E. Bohme. Pp. 151-167. R.4.40. Vol. 66, Part 8: A New Species of Mystacocaridae (Crustacea) from Aloga Bay, South Africa. By A. McLachlan and J. R. Grindley. Pp. 169-175. R.2.10. (Cape Town: South African Museum, 1974.) [1912]

Smithsonian Contributions to Zoology. No. 181: The Costate Species of *Colaspis* in the United States (Coleoptera: Chrysomelidae). By Doris H. Blake. Pp. iii + 24. 65 cents. No. 182: Spider Crabs (Crustacea: Brachyura: Majidae) from the International Indian Ocean Expedition, 1963-1964. By D. J. Griffin. Pp. iv + 35. 85 cents. (Washington, DC: Smithsonian Institution Press, 1974. For sale by US Government Printing Office.) [231]

Australian Academy of Science. Year Book, July, 1974. Pp. 142. (Canberra City: Australian Academy of Science, 1974.) [2312]

Centre National pour l'Exploitation des Océans (CNEXO). Publications. Serie: Actes de Colloques. Colloque sur l'Aquaculture. 22-24 Octobre, 1973. Brest. Pp. 472. Serie: Rapports Scientifiques et Techniques. No. 18: Cartographie Mensuelle des Données sur l'Effort et les Prises de la Pêcherie Palangrier Thonière Japonaise de l'Océan Atlantique, 1956-1973. Par J. Yves Le Gall. (Brest: CNEXO, 1974.) [231]

On the Safety of Disposing of Radioactive Waste in the Asse Salt Mine. Pp. 48. (München: Gesellschaft für Strahlen- und Umweltforschung mbH., 1974.) [131]

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Institut pour l'Encouragement de la Recherche Scientifique dans l'Industrie et l'Agriculture. Rapport Annuel Exercice 1973. Pp. 491. (Bruxelles: Institut pour l'Encouragement de la Recherche Scientifique dans l'Industrie et l'Agriculture, 1974.) [301]

Canada. Department of Energy, Mines and Resources. Bulletin 227: The Bennett Lake Cauldron, Subsidence Complex, British Columbia and Yukon Territory. By M. B. Lambert. Pp. xviii + 213. \$6. Paper 73-31: Sedimentary History and Tectonic Evolution of the Sustut and Sifton Basins, North Central British Columbia. By Gerhard H. Eisbacher. Pp. 57. \$3.50. (Ottawa: Information Canada, 1974.) [3012]

The Art of Managing the Environment. (A Ford Foundation Report.) Pp. 42. (New York: Ford Foundation, 320 East 43 Street, 1974.) [30]

United States Department of the Interior: Geological Survey. Professional Paper 845: Ammonites from the Navesink Formation at Atlantic Highlands, New Jersey. By William A. Cobban. Pp. iii + 21 + 1 plates. (Washington, DC: Government Printing Office, 1974.) \$1.35. [301]

National Research Council Canada. Annual Report on Scholarships and Grants in Aid of Research, 1973/74. Pp. xi + 560. (Ottawa: Research Council Canada, 1974.) \$2.50. [301]

The American Journal of Drug and Alcohol Abuse. Vol. 1, No. 1, 1974. Pp. 1-140. Subscription rate for Vol. 1 (3 issues). \$25 + \$2.55 postage outside USA. (New York: Marcel Dekker, Inc., 1974.) [301]

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